DEPARTMENT FOR SCIENTIFIC PROGRAMMING AND INCENTIVE ACTIONS

# Incentive programs at the Institut Pasteur

Examples of the Inter-Pasteurian Concerted Actions (ACIP) and Transversal Research Programs (PTR) • December 2023





# Introduction to Incentive Programs at the Institut Pasteur

Founded in 1887 and based in Paris, France , the Institut Pasteur is a private , non -profit foundation with four missions of public interest : research , public health , education , and the identification of research applications.

Today, the Institut Pasteur is connected worldwide through the Pasteur Network (PN), a global research community of more than 30 members located in 25 countries across 5 continents. PN represents a unique asset in the world for interdisciplinary biomedical research and education.

Within its Scientific Division, Institut Pasteur dedicated a whole department to implement the key ambitions of its strategic plan : the Scientific Programming and Incentive Actions Department (SPAIS). The SPAIS oversees an ecosystem of "Incentive Actions ", which are tools for steering and deploying Institut Pasteur's scientific vision. Internal calls for projects are launched every year, with the aim of selecting - thanks to evaluation and selection committees made up of international experts - projects of excellence, emblematic of Pasteurian research.

With their cutting -edge approaches, these rigorously selected multi /interdisciplinary projects constitute a solid portfolio of innovations and skills, with multiple objectives for Institut Pasteur:

- **To develop transversality** between the different research entities of the Institut Pasteur in Paris and within the Pasteur Network.
- To match recent societal challenges by encouraging multidisciplinary research between the Campus teams
- To contribute to major advances in knowledge and public health issues.

The SPAIS aims to build a robust portfolio of **multi** -/interdisciplinary and innovative research **projects**, and to make them attractive for further development by partners/funders (public, private, industrial, or philanthropic).

The SPAIS closely collaborates with other administrative and technical support departments in managing various Incentive Programs, including focused ones aimed at strengthening collaboration between Pasteur Network teams: ACIP (Inter-Pasteurian Concerted Actions), and PTR (Transversal Research Programs).

• ACIP calls aim to support research projects focused on a public health topic with funding of up to €50,000 for a maximum period of 2 years.

• **PTR calls** aim to initiate exploratory research projects that are ambitious, innovative, and interdisciplinary with funding of up to €250,000 for a maximum period of 24 months.

These two annual calls for proposals follow a well-defined process, from project submission to selection.

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Submissions are made online through a dedicated platform that establishes a direct link between scientists from the Institut Pasteur and PN, and the SPAIS. Projects are evaluated by international peer reviewers before being selected for funding by a selection committee.

This book focuses on the ACIPs, and PTRs selected by the Institut Pasteur:

• The projects selected in the last past call (2023-2024)

• In the second part, short summaries of previously funded projects since 2014 in Annexes

## INCENTIVE PROGRAMS KEY NUMBERS

**PTRS** Number of PTR projects

funded since 2019

**YEARS** 

Maximal duration of the ACIPs and PTRs funding

ACIPs Number of ACIP projects funded since 2010 228

50k€ ACIP funding

# 250k€ PTR funding

PASTEURIAN COLLABORATIONS

Number of collaborations for ACIPs and PTRs since 2019

# Image: Constraint of the state

# INSTITUTES

Number of Pasteur Network institutes across the 5 continents, including Institut Pasteur Paris, involved in PTRs and ACIPs since 2019.

# DEPARTMENT OF SCIENTIFIC PROGRAMMING AND INCENTIVE ACTIONS – SPAIS



# SUMMARY

### PORTFOLIO OF ACIP PROGRAMS FUNDED IN 2023

### ACIP CALL 2023

**Keywords:** Zoonosis, Leptospirosis, bacteria, immunity, immunopeptidome, dendritic cell (DCs), vaccine, lipopolysaccharide (LPS), immunopeptidomics, One Health, CD4+, immune answer, protein design, mass spectrometry, recombinant protein

### Strategy for genomic surveillance of pathogens in wastewater

An Early Warning System for AIV Transmission and Gene Flow in Cambodia ACIP n°676 – 2023 AVERT-Cam p06 Keywords: Genetic epidemiology, bioinformatics, avian influenza virus, one health, zoonosis, meta-analysis, avian, bird, poultry, genomics, review, AIV, H5N1, phylodynamic, surveillance, poultry, transmission,maintenance, emerging disease

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**Keywords**: Tick-borne diseases, TBD, emerging diseases, vector, Vector Borne Disease, VBD, One Health, tick, climate, epidemiology, meta-analysis, zoonosis, Crimean-Congo hemorrhagic fever virus, CCHFV, orthonairovirus, preparedness, ruminants, sequencing, serology

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### Prediction of human rabies based on blood biomarkers

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### **PTR CALL 2023**

### Miniature polymerases of archaeal viruses: a new class of enzymes with high biotechnological potential

Tackling antifungal resistant Aspergillus fumigatus with surfactant protein D PTR n° 617 – 2023 ANTIFUN p16 Keywords: Aspergillus fumigatus, antifungal, antifungal resistance, AMR, genetic diseases, invasive fungal, disease, treatment

### Auditory neuropathies: new genetic models and audiological tests to decipher the underlying pathophysiological mechanisms

PTR n° 618 – 2023 NeuropatHEAR p18 Keywords: Brain Connectivity Diseases and Neurodegenerative Diseases, hearing, diagnostic, model, re-emerging auditory brainstem responses, hearing deficit, transcriptomics, neural cell function, communication and signaling, neurotransmission, sensory system

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**Keywords:** Shigella, iron sulfur clusters, biomarker, bacteria, polymorphism, gene decay, evolution, Fe-S, SUF,ISC, phylogeny, genomics, stress resistance

### Structural membrane dynamics and enzymatic activity of lipoprotein modification enzyme Lgt in the presence of peptide inhibitors

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**Keywords**: AMR, vaccine, cytokine, immunology, intracellular bacteria, host defense, macrophage, Legionella pneumophila and Salmonella enterica Typhimurium, metabolomics, immune-phenotyping, computational analysis, drug, mitochondria

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**Keywords**: Dengue, biomarker, functional genomics, transcriptomics, immunity, cytokine, single cell, population genetics

### A quest for atomic-level description of translation factor interactions exclusive from trypanosomatids

#### 

Keywords: Bacteriophage, biosafety, pneumonia, chronic infection, antimicrobial,

nosocomial infection, therapy, preclinical, therapy, quality control

### Role of human auto-antibodies against nicotinic acetylcholine receptors in psychiatric disease

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**Keywords**: T7SSb, Streptococcus gallolyticus subsp. gallolyticus, emergence, bacteria, TelE, microbiota, gut colonization, cancer, LXG-effectors

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### PORTFOLIO OF INTER-PASTEURIAN CONCERTED ACTIONS (ACIP) CALL 2023





First investigation of Leptospira immunopeptidome associated with dendritic cells presentation: towards a cross-protective sterilizing vaccine against leptospirosis Project ACIP n° 623- 2023 LEPTIDOME



### SCIENTIFIC COORDINATOR

**Dr. Julie CAGLIERO**, Leptospirosis Research and Expertise unit, Institut Pasteur Noumea, New Caledonia

### PASTEUR NETWORK COLLABORATORS

Dr Catherine WERTS – Institut Pasteur Paris, France Dr Rosario DURAN- Institut Pasteur Montevideo, Uruguay Dr. Alejandro BUSCHIAZZO- Institut Pasteur Montevideo, Uruguay

### **PROJECT SUMMARY**



### **KEYWORDS**

Zoonosis, Leptospirosis, bacteria, immunity, immunopeptidome, dendritic cell (DCs), vaccine, lipopolysaccharide (LPS), immunopeptidomics, One Health, CD4+, immune answer, protein design, mass spectrometry, recombinant protein



### THE LEPTOSPIROSIS

Leptospirosis is a worldwide bacterial disease that affects both humans and animals, particularly in tropical areas. It is caused by bacteria of the genus *Leptospira*. Rodents, especially rats, are the main reservoirs. They excrete the bacteria in their urine. The incubation period is generally between 4 and 14 days for humans. Symptoms of leptospirosis are highly variable, ranging from mild flu-like symptoms to serious complications: kidney failure, hemorrhagic syndrome, neurological disorders and even death. This wide range of symptoms complicate the diagnosis. However, rapid diagnosis is crucial to initiate treatment, as only early administration of appropriate antibiotics can effectively treat the disease but may fail to eradicate renal colonization.

### LIMITED VACCINAL SOLUTION

Vaccines against human leptospirosis are approved in very few countries, with suboptimal efficacy: based on killed bacteria, the triggered humoral response is mainly directed against the bacterial lipopolysaccharide (LPS), ultimately resulting in limited protection, and only restricted to the vaccine serovars from among the >300 serovars reported so far. In this context, **a cross-protective, sterilizing, and safe vaccine against leptospirosis is therefore urgently needed.** 

### THE PROJECT

It remains unknown how Leptospira-exposed immune Dendritic Cells (DCs) activate T-cell (lymphocyte). Indeed, the identity of the leptospiral peptides presented to lymphocytes remains unknown. Interestingly, innovative concepts in the field of next-generation vaccinology are based on mass spectrometry (MS) and structural biology. Identifying the different antigens presented at the dendritic cell surface (the immunopeptidome) is now feasible using Mass spectrometry. The goal of this ACIP is to implement, for the first time, the MS-based investigation of the *Leptospira* immunopeptidome. The consortium will then design vaccine candidates *in silico*, as a first step towards the development of a universal and sterilizing vaccine against *Leptospira* infection.

Leptospirosis causes 1 million severe cases and over 60,000 deaths worldwide each year

Leptospirosis lethality is estimated at 10% of cases



### Strategy for genomic surveillance of pathogens in wastewater

#### Project ACIP nº 648- 2023 CLIMPATHIC



### SCIENTIFIC COORDINATOR

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### PASTEUR NETWORK COLLABORATORS

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### **PROJECT SUMMARY**



### **KEYWORDS**

Norovirus, Dengue, Diarrheal diseases, One Health, Wastewater, Metagenomics, Virus, Fecal-Oral Transmission route, Gastroenteritis, Climate change, Endemic, Emerging Diseases, Reemerging diseases, Pandemic preparedness, Surveillance



### **CLIMATE CHANGE AND (RE)-EMERGENCES**

Climate change, with its rising temperatures and shifting precipitation patterns, has a profound impact on the distribution and transmission of pathogens, which poses a significant threat to public health. Furthermore, warmer and wetter conditions and more extreme weather variations resulting from climate change provide ideal conditions for the growth and spread of foodborne pathogens like norovirus, Salmonella, and Campylobacter.

### (RE)-ERMERGENCES AND MONITORING PUBLIC HEALTH

An effective surveillance system using methods combining PCR, targeted next-generation sequencing and metagenomic approaches is essential to control the spread of endemic pathogens, and anticipate their (re)emergence in populations. Wastewater monitoring has already successfully guided public health decisions during the COVID-19 pandemic or in monitoring hepatitis A and poliovirus propagations. Implementing similar systems for other pathogens, combined with the consideration of environmental changes due to climate change, is an essential approach to proactively responding to infectious disease threats. This global approach will enable us to assess rapidly epidemics and contribute to the preservation of public health.

### THE PROJECT

The goal of this project is to develop tools for genomic surveillance of (endemic, (re)-emerging) pathogens in the context of climate change. The full strategy for the detection of pathogens in wastewater, will be developed for the norovirus and dengue as proof of concept. The norovirus is a highly contagious virus commonly associated with outbreaks of gastroenteritis and is nearly certain to be found in wastewater due to its fecal-oral transmission route. Dengue, on the other hand, is mentioned as important to follow in the context of climate change and can also be found in wastewater. This project's methods and information will be useful to develop an efficient strategy to detect emerging pathogens as well as certain mutations that are for example linked to vaccine escape.

The direct damage costs of climate change to health is estimated to be between USD 2-4 billion/year by 2030. (WHO)

Wastewater surveillance was used in almost 55 countries worldwide to analyze wastewater samples for SARS-CoV-2 RNA from more than 2,690 sites. (FEMS Microbes. doi: 10.1093/femsmc/xtad003)



### AVERT-Cam: An Early Warning System for AIV Transmission and Gene Flow in Cambodia

Project ACIP n° 676- 2023 AVERT\_Cam



### SCIENTIFIC COORDINATOR

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### PASTEUR NETWORK COLLABORATORS

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### **PROJECT SUMMARY**

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### **KEYWORDS**

Genetic epidemiology, bioinformatics, avian influenza virus, one health, zoonosis, meta-analysis, avian, bird, poultry, genomics, review, AIV, H5N1, phylodynamic, surveillance, poultry, transmission, maintenance, emerging disease



### AVIAN INFLUENZA VIRUS: A SERIOUS PUBLIC HEALTH THREAT

Avian influenza viruses (AIV) pose a serious public health threat in Cambodia, with highly pathogenic avian influenza (HPAI) H5N1 viruses and multiple subtypes of low pathogenic avian influenza (LPAI) viruses circulating. H5N1 infection in humans can cause severe disease and has a high mortality rate. Since 2005, Cambodia has reported a total of 58 human cases of H5N1 infection to the WHO, of which 36 were fatal (CFR: 66%). In February and October 2023, Cambodia reported its two first human cases since 2014.

### UNDETERMINED MECHANISMS OF TRANSMISSION AND MAINTENANCE

Human cases of Influenza A (H5N1) infection are often associated with close contact with infected live or dead birds, or contaminated environments. To date, HPAI H5N1 viruses do not efficiently spread from human-to-human, and human infection is primarily associated with poultry contact. According to the WHO, while the virus remains endemic in poultry populations, further human cases can be expected. Despite ongoing surveillance efforts, knowledge gaps remain concerning the factors influencing HPAI virus transmission and maintenance, and the effectiveness of current control measures.

### **THE PROJECT**

This project aims to improve our understanding of AIV transmission and evolution within poultry populations, as well as its potential for spillover to humans. The consortium will develop an early warning system for AIV in Cambodia. This ACIP will identify factors that influence the transmission, maintenance, and diversification of the virus. Moreover, the teams will focus on the gene flow of AIV, specifically its transmission between wild birds, poultry sectors, and humans. Collectively, this data will furnish tools for assessing the effectiveness of AIV control measures. The ultimate goal is to provide evidence-based recommendations for the most effective mitigation strategies against AIV that can be communicated to stakeholders in the poultry industry, public health officials, and policymakers. Since 2003, 873 human cases have been reported with 458 deaths worldwide (WHO).

in 2006, \$45 million in international aid was allocated to Cambodia to combat the major avian flu epidemic then underway



An insight on tick-borne pathobiome in Central African Republic, Ivory Coast and Tunisia Project ACIP n° 692- 2023 TicPath



#### SCIENTIFIC COORDINATOR

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#### PASTEUR NETWORK COLLABORATORS

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### **PROJECT SUMMARY**



### **KEYWORDS**

Tick-borne diseases, TBD, emerging diseases, vector, Vector Borne Disease, VBD, One Health, tick, climate, epidemiology, meta-analysis, zoonosis, Crimean-Congo hemorrhagic fever virus, CCHFV, orthonairovirus, preparedness, ruminants, sequencing, serology

### THE UNPRECEDENTED SPREAD OF TICK

In human health, ticks occupy the second position after mosquitoes and in animal health, the first before mosquitoes as vectors of pathogens. Many tick borne diseases (TBDs) are zoonotic and infection in animals is often asymptomatic. TBDs are recognized as a serious and growing public health issue and a cause of major losses in livestock worldwide. Factor such as human activities (deforestation, land use, habitat fragmentation, etc.), international movement (animal trade, refugees, travelers, etc.), migration of ticks bearing birds from one country to another as well as from one continent to another in addition to micro and macro climatic changes lead to a redistribution and increase of zoonotic infections, both emerging and reemerging worldwide.

### THE TICK-BORNE DISEASE RISK FOR HUMAN HEALTH

Ticks are known as carriers for a wide range of pathogens, including viruses, bacteria, protozoa and helminths. Tick-borne diseases (TBDs)s are emerging and/or reemerging due to the geographical expansion of their tick vectors, notably in north America, Europe, Asia as well as in Japan, where they are responsible for majority of the vector-borne diseases (VBDs). Among them, Bunyaviruses are the most frightening group of emerging TBVs.Crimean-Congo haemorrhagic fever virus (CCHFV) is one of them present in Africa, Asia and Europe. CCHFV affects the nervous system with neurological manifestations, which precede serious hemorrhagic signs with high case fatality rates (up to 40%) in humans. There is a lack of vaccine as well as specific treatment, so it would be crucial to develop research themes in public health to guide preventive strategies but also those of management of possible epidemics of TBDs.

### **THE PROJECT**

This project aims to evaluate the risk of TBDs of medical interest, with a special focus on the circulation of the different arboviruses, bacteria, and parasites associated to human health in the Central African Republic, Ivory Coast and Tunisia by using new high throughput approach able to identify a wide range of tick-borne pathogens. The consortium will take advantage of existing databases and collection and will update TBDs common knowledges on detection and ruminants sero-prevalence. The ultimate goal of this project is to map the tick and TBDs risk to manage better healthcare decisions and preparedness.



According to climate projections, the distribution of ticks will progress northwards by 35 to 55 km per year. (doi: 10.1111/j.1365-2664.2012.02112.x)

CCHFV infection can occur in human by direct tick's bite, contact with infected animal tissues or through nosocomial routes.



Genomic epidemiology of Acinetobacter spp. and phylodynamics of resistance spread Project ACIP n° 702- 2023 ACIPhylo



#### SCIENTIFIC COORDINATOR

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#### PASTEUR NETWORK COLLABORATORS

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### **PROJECT SUMMARY**



### **KEYWORDS**

Bacteria, *Acinetobacter baumannii,* multidrug resistance, One Health, *Acinetobacter spp.*, plasmid, nosocomial, phylogeny, resistance gene, virulence factor, metagenomic



### ACINETOBACTER spp

Acinetobacter spp. are gram-negative, opportunistic and nosocomial bacteria. Acinetobacter spp. can be isolated from various habitats such as soil, sediment surface or wastewater. Side to humans, *Acinetobacter spp.* have been isolated from various species, including cattle, pigs, horses, dogs, cats, birds and reptiles. These bacteria can cause pneumonia, sepsis, meningitis, mastitis, bronchopneumonia, urinary tract and wound infection... 80% of *Acinetobacter spp.* human infections are caused by the solely *Acinetobacter baumannii*. Infections can be therapeutically challenging, and the mortality rate of *Acinetobacter baumannii* varies from 19% to 54% depending on the severity of the infection and the patient's underlying health conditions:.

### A.BAUMANNII'S MULTIDRUG RESISTANCE AND HUMAN HEALTH

The World Health Organization (WHO) declared that A. *baumannii* is one of the most serious of the "ESKAPE"<sup>1</sup> list. Indeed, *A. baumannii*'s main features is its ability to adapt and resist to antibiotic pressures. A. *baumannii* has a huge propensity to be multidrug-resistant or extensively drug-resistant. Indeed, this bacteria can change up to 3% of its genome to acquire multidrug-resistance genes. Today, *A. baumannii* is resistant to most first-line antibiotics. Early identification of patients exposed to risk factors favors the effectiveness of therapeutic outcome. *Acinobacter spp.* diversification is partly linked to changes in habitat. Combine with its presence in livestock, veterinary and human medicine, the integrated "One Health" approach, currently poorly documented for *Acinetobacter spp.* genus, could be particularly relevant to fight against this pathogen.

<sup>1</sup> The most common multidrug-resistant pathogens are grouped in the acronym "ESKAPE": *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter cloacae* 

### THE PROJECT

This work will provide the first data on Acinetobacter mapping in 3 French regions. Firstly, the consortium will sample natural and hospital areas to detect and sequence *Acinetobacter spp.* strains present in humans and the environment. Sequencing will enable researchers to perform comparative analyses to identify and annotate *Acinetobacter spp's* antimicrobial resistance genes and virulence factors. This work will give access to a comparative approach to *Acinetobacter spp* strains, enabling an integrated understanding of the phylogeny of these bacteria, and hence their dynamics of acquisition of antibiotic resistance genes, especially in the hospital environment.

A. baumannii account for 2% of all healthcareassociated infection in USA and Europe.

45% of *A. baumannii* isolates are multi-drug resistant



### Prediction of human rabies based on blood biomarkers

### Project ACIP n° 711- 2023 PrediRab



### SCIENTIFIC COORDINATOR

Dr. Laurent DACHEUX, ERI – Institut Pasteur Paris, France

### PASTEUR NETWORK COLLABORATORS

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- Dr. Edgard Valéry ADJOGOUA Institut Pasteur Abidjan, Ivory Coast
- Dr. Perrine PARIZE- Institut Pasteur Paris, France
- Dr. Sandrine AROS Institut Pasteur Paris, France
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### **PROJECT SUMMARY**



CSF: Cerebrospinal fluid

### **KEYWORDS**

Rabies, virus, biomarkers, emerging infectious disease, EID, proteomics, metabolomics, healthcare monitoring, zoonosis, POCTs, prophylaxis, immunoassay, diagnosis, mass spectrometry, biocollection, blood, CSF



### **RABIES: STILL A HUMAN HEALTH THREAT**

Rabies is a zoonotic, vaccine-preventabl, viral disease affecting potentially all mammals, including humans, in over 150 countries and territories. Once clinical symptoms have appeared (central nervous system infection), rabies is always fatal. To date, this viral disease is still responsible for an estimated 60,000 human deaths per year: 40% of victims are children under 15 year-old. In 99% of cases, death is due to dog-mediated rabies. Prototype of neglected infectious diseases, it mainly affects the poorest populations in rural areas of low- and middle-income countries, mainly in Asia and Africa (95% of the victims). However, the real burden of human rabies remains largely underestimated so far.

### AN UNDERESTIMATED PUBLIC HEALTH ISSUE

One of the main reasons for the underestimating of the rabies burden is the difficulty of confirming the diagnosis. This situation is mainly due to the fact that populations most affected are poorly equiped and informed about the disease. Moreover, diagnosis relies exclusively on laboratory analysis of specific biological samples (saliva, skin biopsy and/or brain biopsy). As a result, biological diagnoses, which are all too rarely carried out, are generally limited to geographical areas withi central laboratories acting as national reference centers for rabies. This virtual centralization of cases has a considerable impact on the effectiveness of rabies surveillance protocols. Indeed, most human cases occur in rural areas, and therefore go undiagnosed due tolack of access to suitable laboratories and/or difficulties in transporting samples (delays, preservation of the cold chain, etc.). Numerous studies have attempted to better estimate the burden of rabies using indirect methods, but more and reliable direct detection and surveillance are necessary gor effective rabies control.

### THE PROJECT

The aim of this project is to identify biomarkers of rabies in blood samples. The teams will conduct complementary omics strategies applied to prospective cohorts of biologically confirmed encephalitis (rabid and nonrabid) and control patients in Algeria and Côte d'Ivoire. This project will pave the way for the development of new, accessible devices to efficiency guide physicians faced with suspected cases of human rabies from conventional blood samples. Rabies is estimated to cost \$8.6 billion a year.

In 2013, Sambo et al. demonstrated that the estimated incidence of mortality in Tanzania was 10 to 100 times higher than officially reported. Sambo et al. (2013) https://doi.org/10.1371/jour nal.pntd.0002510

### PORTFOLIO OF TRANSVERSAL RESEARCH PROGRAMS (PTR) CALL 2023



### Miniature polymerases of archaeal viruses: a new class of enzymes with high biotechnological potential

Project PTR n° 614- 2023 MINIVIRPOL



### SCIENTIFIC COORDINATOR

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### PASTEUR NETWORK COLLABORATORS

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### **CONFIDENTIAL PROJECT**



### Tackling antifungal resistant *Aspergillus fumigatus* with surfactant protein D

Project PTR n° 617- 2023 ANTIFUN



### SCIENTIFIC COORDINATOR

**Dr. Vishukumar AIMANIANDA**, Immunobiology of Aspergillus Group - Institut Pasteur Paris, France

#### PASTEUR NETWORK COLLABORATORS

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### **PROJECT SUMMARY**

Note: X & Y stand for the actual names of compounds studied



### **KEYWORDS**

Aspergillus fumigatus, antifungal, antifungal resistance, AMR, genetic diseases, invasive fungal disease, treatment



### THE OVERLOOKED PATHOGENIC-FUNGI BURDEN

Our perception about fungi is limited to edible-mushrooms although hundreds of microscopic-fungi cause fatal invasive fungal diseases (IFD). Annually, pathogenic-fungi kill more than the deaths caused by malaria, nevertheless, IFD are overlooked. On the other hand, antifungal drugs available to treat IFD are limited, and the mortality rate associated with IFD remains high even after antifungal treatment. This poor prognosis is due to diagnostic delay lowering antifungal efficacy and antifungal resistance acquired by fungal pathogens. Therefore, there is a need to overcome these antifungal-associated issues to achieve better prognosis for IFD.

### INVASIVE ASPERGILLUS: A FUNGAL-DISEASE OF INTEREST

Among IFD, invasive *aspergillosis* (IA) caused by pathogenic species of *Aspergillus* is of global concern. *Aspergillus fumigatus* is an opportunistic pathogen, and the major pathogenic *Aspergillus* species responsible for invasive *aspergillosis* (IA), worldwide.

Azole drugs are the first line of antifungals for IA-treatment. They target lanosterol-14-demethylase, an essential enzyme in fungal membrane constituent. Nonetheless, the last decade has experienced the emergence of azole-resistance in *A. fumigatus*; of threat is the pan-azole resistance due to specific point mutations in the dedicated *A.fumigatus* DNA sequence. Agricultural application of imidazole fungicide and drugpressure due to long-term treatment with azoles in chronically infected patients are the main reasons for the emergency of azole resistance. Therefore, alternative strategies are essential to treat invasive fungal disease caused by azole resistant *A. fumigatus*.

### THE PROJECT

The consortium identified a protein (X), present in the human and having anti-*A. fumigatus* activities with distinct targets from the Y counpound, which is known to have an inhibitor effect on Candida spp. First, the teams will focus on understanding the anti-*Aspergillus fumigatus* mechanism associated with X and Y. This project will decipher antifungal potential of these two compounds, individually and in combination, against azole sensitive as well as azole-resistant isolates of *A. fumigatus*. Then, researchers will validate activities of X and Y compounds *in vivo*, using mouse model.

Annually, pathogenicfungi kill 1.6-million people globally.

Aspergillus fumigatus is responsible for more than 80% of human cases of aspergillosis.



# Auditory neuropathies: new genetic models and audiological tests to decipher the underlying pathophysiological mechanisms

Project PTR n° 618- 2023 NeuropatHEAR



#### SCIENTIFIC COORDINATOR

**Dr. Nicolas MICHALSKI,** Plasticity of Central Auditory Circuits – Hearing Institute, Paris, France

### PASTEUR NETWORK COLLABORATORS

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### **PROJECT SUMMARY**

Note: "Gene X" stands in place of the real name of the studied gene.



### **KEYWORDS**

Brain Connectivity Diseases And Neurodegenerative Diseases, hearing, diagnostic, model, re-emerging auditory brainstem responses, hearing deficit, transcriptomics, neural cell function, communication and signaling, neurotransmission, sensory system



### HEARING DEFICIT IMPACT ON SOCIETIES

Hearing deficits significantly impact global public health, affecting over 6% of the world population and projected to impact over 700 million people by 2050 (WHO). The impact on social well-being is profound, with hearing loss linked to communication difficulties, cognitive impairment, delayed language development in children, and increased risk of mental health issues such as dementia and depression. Addressing hearing impairments is crucial for enhancing overall quality of life and mitigating the broader public health implications associated with these sensory deficits. The majority of these deficits stem from dysfunctional auditory sensory cells, particularly hair cells, resulting in abnormal auditory thresholds. However, there is a growing recognition that deficits in the auditory nerve, known as auditory neuropathy, contribute substantially to hearing impairments, accounting for at least 10% of cases.

### AUDITORY NEUROPATHIES

Auditory neuropathies encompass a range of congenital and acquired disorders disrupting sound transmission from the inner ear to the brain, affecting the encoding of temporal fine structures and sound intensities. Individuals with neuropathies often have near-normal auditory thresholds but struggle to understand speech, especially in noisy environments, leading to social challenges. Additionally, patients may experience auditory distortions like tinnitus and hyperacusis, reducing tolerance for moderate sound levels. Despite the severity of disabilities caused by auditory neuropathies, the field lacks preclinical animal models reliably mimicking the human pathology, precluding the identification of neuropathy markers for diagnosis, the deciphering of the underlying pathophysiological mechanisms and involved neuronal circuits, as well as the development of treatments.

### THE PROJECT

The consortium aims to develop reliable models to mimic the human pathology to decipher its underlying mechanisms, identify biomarkers and develop new diagnosis tools. Researchers will develop a new cutting-edge non-invasive audiological test, which specifically probes the activity of subtypes of auditory nerve neurons. The teams will also provide the first genetic model for deficits of subtypes of auditory nerve neurons. Using recently developed single cell spatial transcriptomics methodologies, the consortium will decipher the auditory circuits activated by each neuron subtype of the auditory nerve. Hearing impairments will be the seventh leading cause of burden of disease by 2030.



### Iron-Sulfur Clusters Biogenesis Polymorphism and Virulence in Shigella

### Project PTR n° 619- 2023 ShigeFeS



### SCIENTIFIC COORDINATOR

Dr. Sarah DUBRAC, SAMe - Institut Pasteur Paris, France

### PASTEUR NETWORK COLLABORATORS

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### **PROJECT SUMMARY**



### **KEYWORDS**

*Shigella,* iron sulfur clusters, biomarker, bacteria, polymorphism, gene decay, evolution, Fe-S, SUF, ISC, phylogeny, genomics, stress resistance



### SHIGELLOSIS: A BACTERIAL DISEASE

Shigellosis stands as a spectrum of diarrheal disorders, varying in intensity from mild to severe manifestations. The symptoms involve abdominal pain, fever, and profuse diarrhea, often with traces of blood and mucus. This disease is endemic in developing countries, exploiting the fecal-oral transmission route, facilitated by poor-hygiene and contamination of food and water sources. Shigellosis is caused by Shigella bacterial genus, comprising four distinct species: *Shigella sonnei*, *Shigella flexneri*, *Shigella boydii, and Shigella dysenteriae. Shigella sonnei* is the most common species worldwide, mainly causing the milder forms of shigellosis; while *Shigella flexneri* is the most virulent protagonist, carrying a fatality rate up to 20%.

Shigellosis raised public health attention due to the escalating emergence of multi-resistant Shigella strains in annual reports.

### AN INTRUIGUING GENETIC EVOLUTION

Fe-S clusters stand as one of the most ancient inorganic cofactors, they are essential for all living cells. Fe-S are ubiquitous over a large array of biological contexts in Shigella: bioenergetics, gene modulation, metabolic orchestration, and the intricate pathways of biosynthesis within bacterial domains. Shigella, as all enterobacteria, has two Fe-S production pathways named ISC and SUF. ISC is the housekeeping system, whereas SUF is only active under stress conditions encountered by Shigella throughout infection. Usually, such an ubiquitous system is highly conserved. But, among Shigella genus, the consortium identified an unexpected polymorphism within the DNA locus governing SUF. This polymorphism was obtained through an evolutive mechanism named "gene decay" and its evolutive advantage is intriguing in the context of a bacterial pathogen.

### THE PROJECT

By focusing on the essential central process Fe-S based biology, this project will provide a molecular, metabolic, evolutive, and cellular characterization of the virulence of Shigella strains. This project aims at exploiting Shigella biodiversity to investigate evolution of Fe-S cluster biogenesis systems and their impact on virulence. Based on consortium's preliminary data, the project will (1) recapitulate how Fe-S biogenesis gene decay arose in Shigella populations; (2) study functional consequences of the inactivation of the SUF biogenesis system, thought to be important for Shigella infection (3) assess the impact of gene decay within Fe-S biogenesis factors on Shigella virulence and on stress adaptation

Shigellosis is estimated to cause 200 000 deaths each year.



Structural membrane dynamics and enzymatic activity of lipoprotein modification enzyme Lgt in the presence of peptide inhibitors

Project PTR nº 626- 2023 ENDPOINT



### SCIENTIFIC COORDINATOR

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### PASTEUR NETWORK COLLABORATORS

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### **PROJECT SUMMARY**



### **KEYWORDS**

AMR, Acinetobacter, *Escherichia coli, Helicobacter pylori, Pseudomonas aeruginosa,* membrane, bacteria, lipoprotein, post-translational modification, Lgt, HDX MS, mass photometry



### THE ANTIMICROBIAL RESISTANCE'S BURDEN

Antimicrobial resistance (AMR) is on the top 10 of global health concern with significant implications for health, food security and development. Bacteria, viruses, fungi, and parasites have undergone transformative adaptation, reshaping themselves into resistant stains towards current medicinal interventions. This situation is the result of a confluence of factors encompassing the inappropriate and excessive used of antibiotics and the absence of novel antimicrobial breakthroughs. The consequences of AMR are severe, and include higher mortality rates, increased healthcare costs, decreasing quality of life...

Therefore it is crucial to adress this issue.

### HOW ANTIMICROBIAL RESEARCH CAN EVOLVE?

The World Health Organization (WHO) recently pinpoint a list of priority of concern pathogens: the so-called ESKAPE list. The majority of these bacteria have cell envelopes composed of two membranes (diderm). Antibiotic treatment of these species is particularly difficult due to poor entry of molecules into the pathogens cells that have a robust efflux mechanisms of drugs and intrinsic resistance to antibiotics. In general, two basic approaches are used in antibiotic discovery: phenotypic and target-based screening. Both approaches depend on large compound libraries, composed of small chemical molecules or natural products that either inhibit bacterial cell growth, or inhibit the function or enzymatic activity of a crucial pathogen's protein. The use of unexplored biochemical pathways as targets for novel antibiotics is one way to combat AMR.

### THE PROJECT

The concortium has access to structural and functional data of a lipoprotein modification enzyme (Lgt) that is essential for viability and pathogenicity of diderm bacteria. The teams will explore the potential of specific inhibitors of Lgt using combination of their expertise in biochemistry, biophysics, molecular dynamics, chemical biology and peptide chemistry. The ENDPOINT project has the objective to find in Lgt a novel target for broad-spectrum antibacterial agent. In this context, they will describe the molecular involvement of Lgt in the pathogens, and check if it is conserved among bacteria. Then, they will look for stable peptides able to inhibit Lgt activity to open new route for new treatments.

WHO estimate that 700 000 deaths are annually dued to AMR.

The worldwide economic cost of AMR by 2050 is estimated at \$100 trillion.



### Using Comparative Patho-Metabolomics To Decipher How Intracellular Bacteria Subvert Macrophage Immuno-Metabolism Project PTR n° 651- 2023 METABACT



### SCIENTIFIC COORDINATOR

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### **PROJECT SUMMARY**



\* immune cells that fight infection

### **KEYWORDS**

AMR, vaccine, cytokine, immunology, intracellular bacteria, host defense, macrophage, *Legionella pneumophila* and *Salmonella enterica* Typhimurium, metabolomics, immune-phenotyping, computational analysis, drug, mitochondria,



### BACTERIA OF INTERREST ON HUMAN HEALTH

Legionella pneumophila and Salmonella enterica serovar Typhimurium are two bacteria with significant impacts on human health though distinct modes of transmission. Legionella pneumophila causes legionellosis, commonly known as Legionnaires' disease. This severe form of pneumonia is transmitted through the inhalation of contaminated aerosols, from water systems like cooling towers, hot tubs, and plumbing systems. Legionellosis exhibits severe respiratory symptoms, including high fever, cough, and breathlessness, and in some cases, it can be fatal. Salmonella Typhimurium, conversely, is a major contributor to foodborne illnesses in humans: undercooked poultry, eggs, and unpasteurized dairy products. Infection leads to gastroenteritis, characterized by symptoms like diarrhea, abdominal cramps, vomiting, and fever. While most Salmonella infections resolve on their own, severe cases can result in bloodstream infections needing urgent medical intervention.

### LEGIONELLA PNEUMOPHILA AND SALMOLELLA TYPHIMURIUM: INTRACELLULAR BACTERIA

Legionella pneumophila and Salmolella Typhimurium are intracellular bacteria. Intracellular bacteria have evolved to live and replicate within host cells. Because they can only take nutrients from the infected cells, to grow in these host cells intracellular bacteria have to hijack the metabolic pathways of infected cells. The intracellular niche provides also protection against immune system surveillance and antibiotics, making them challenging to eliminate. Understanding the strategies that intracellular bacteria employ to evade the immune system and manipulate host cellular processes is essential for developing effective treatments. Moreover, Legionella pneumophila and Salmolella Typhimurium infect macrophages during disease progression. Macrophages are immune cells in charge of fighting infection and they reprogramm their metabolism to exert their antibacterial actions. Therefore, Legionella pneumophila and Salmolella Typhimurium are particularly relevant organisms to elucidate the impact of various virulence strategies on bacterial growth inside macrophages, such as nutrient salvaging, metabolic interactions with macrophage mitochondria, or the regulation of autophagy during intracellular infections.

### THE PROJECT

The main objective of the project is to identify which metabolic pathways are specifically used by intracellular bacteria to grow inside macrophages. To accomplish it, the teams will analyze and compare the immunometabolism and phenotype of human macrophage infected with *Legionella pneumophila* or *Salmolella typhimurium*. Thanks to this strategy, they will be able to identify common mechanisms across the intracellular bacteria, and they will also be able to pinpoint specific'onesof each bacteria during the infection. This project will allow gaining important new, fundamental knowledge on the host response to infection, but it is also pivotal for the finding of new antimicrobial strategies to fight infection by intracellular bacteria, a research area gaining importance given the unpreceded increase of antibiotics resistance of many bacterial pathogens.

Legionella death rates vary from 5% to 10% in general population and can rise up to 80% in case of untreated immunesuppression patients.

Approximately 1 billion people are at risk of infection with obligate intracellular bacteria.



### Genetic drivers of anti-dengue immune response in Oceanian populations

Project PTR n° 653- 2023 GEDIPOP



### SCIENTIFIC COORDINATOR

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### **PROJECT SUMMARY**



### **KEYWORDS**

Dengue, biomarker, functional genomics, transcriptomics, immunity, cytokine, single cell, population genetics



### DENGUE VIRUS: A MAJOR THREAT TO HUMAN HEALTH

Dengue virus (DENV) is an arbovirus transmitted to humans by the bite of an infected mosquito from the genus *Aedes*. Dengue has been listed by the World Health Organization as one of the 10 major health threats to the human population. There is currently no effective anti-viral therapy targeting DENV and only two vaccines are licensed.. Dengue's clinical presentation varies from asymptomatic to severe cases, with symptoms like sudden fever, nausea, vomiting, muscle and joint pain, and sometimes a rash. Severe dengue can lead to complications like plasma leakage and organ failure. Our understanding of drivers of dengue clinical spectrum is still incomplete. Several factors including virological, clinical, immunological as well as human genetic features may condition the susceptibility to symptomatic dengue and dengue clinical presentation. While our understanding of these factors is incomplete, studying them could help develop adapted prevention strategies and tailored medical care for dengue.

### POPULATION GENETICS: A POTENTIAL DRIVER OF ANTI-DENGUE IMMUNE RESPONSE

Pacific Islanders have borne the brunt of dengue virus infections since it was imported in the 19th century, and turned persistent since the Second World War. Today, this region is a hot spot for dengue virus transmission. In New Caledonia (NC), a French overseas territory in the South Pacific region, dengue outbreaks have become more frequent over the past decade. These outbreaks involved several DENV serotypes and resulted in more severe cases over the last outbreaks. For example, in 2013, the DENV epidemic in NC recorded over 10,000 cases, 5 deaths and a cost of 13 million euros. Northeastern NC, which is mostly populated by Melanesians, exhibits a higher dengue indidence. Studies have been carried out to identify the genetic variants and their biological mechanisms linked to the occurrence of symptomatic and severe dengue. However, the limited inclusion of Oceanian populations in these studies limits their potential benefit for Pacific Islanders, even though they are particularly at risk.

### THE PROJECT

The teams hypothesize that the specific demographic and adaptive history of Oceanian populations has shaped their present-day genetic background that, in turn, contributes to variation in immune responses and differences in susceptibility to certain viral infections, and especially dengue. The main objective of the project is to characterize Oceanian populations' anti-viral immune response and identify its genetic drivers. Dengue is an epidemiologically relevant disease in the context of NC, where present-day populations recapitulate the genetic diversity of the South Pacific region.

The project will combine genomic and immunologic approaches to characterize the genetic drivers of anti-dengue innate immune response of 100 individuals of Melanesian ancestry and 100 individuals of European ancestry in New Caledonia. For the first time, this study will identify how Oceanians genetic uniqueness regulates immune response to a virus threatening their health. Knowledge generated will inform Public Health strategies aiming at limiting the risk and improving the management of dengue in the Pacific region through targeted information campaigns, prevention and screening, ultimately mitigating Oceanian populations vulnerability to dengue.

DENV is present over 128 countries

In 2013, DENV seroprevalence in New-Caledonia was estimated at 60%



### A quest for atomic-level description of translation factor interactions exclusive from trypanosomatids

### Project PTR n° 656- 2023 TraFITryp



#### SCIENTIFIC COORDINATOR

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### **PROJECT SUMMARY**



### **KEYWORDS**

Chagas Disease, *Trypanosoma cruzi*, trypanosomatids, translation, protein structure, crystallography, NMR, HDX-MS, Mass Spectrophotometry



### **CHAGAS DISEASE**

Chagas disease, Leishmaniasis, and Human African Trypanosomiasis, caused by trypanosomatids, pose significant global health challenges lacking preventive vaccines. Notably, Chagas disease, caused by Trypanosoma cruzi, persists globally, with limited treatment options (benznidazole and nifurtimox) facing ongoing studies due to serious side effects. The estimated 6-7 million infections in the Americas and globalization of Chagas disease put it as a major public health issue, requiring more research effort for the development of new therapeutic strategies. In the context of Chagas disease, which presents various transmission routes and diverse manifestations, there is a critical need for specific treatments, especially for the disease's chronic phase. Despite recent efforts, large-scale screenings have yet to produce effective inhibitors with fewer side effects. Trypanosomatids' unique molecular and cellular features offer potential for selective inhibition. However, to uncover this potential, the molecular mechanisms specific to trypanosomatids must be reconstituded and described at atomic resolution, which provides the fundamental information on the mechanistic differences between the translation machinery of the trypanossomatids and of their mammalian hosts.

### **SPECIFIC TARGET**

Protein synthesis is essential for growth and proliferation of all cells. The process for protein synthesis (or translation) can be described in three stages, translation initiation, elongation and termination. Translation initiation in eukaryotes is a complex process dependent on the action of several transient protein factors. The interaction between these protein synthesis factors and between them and the messenger RNA (mRNA) are essential for promoting and regulating the global process of protein synthesis. The protein synthesis machinery of trypanosomatids presents several key differences as compared to mammalian cells. These differences include the presence of an hypermodified molecule at the 5'-end of all mRNAs, the higher number of genes encoding translation initiation factors of mRNAs and the mode of interaction between the translation initiation factors. These core differences are essential for the parasites' survival, they are therefore good candidates to be explored for selective inhibition for the development of new compounds against pathogenic tripanosomatids.

### **THE PROJECT**

Through a multidisciplinary approach, the consortium aims to reconstitute and determine the molecular mechanisms of the trypanosomatid-specific translation initiation machinery. The major aim of this proposal is to study the structure and molecular association of trypanosomatids EIF4E translation factors with their different interacting partners (protein/RNA) in the protein synthesis machinery. Specific chemical tools will be developed for 1) isolation of stage specific translation initiation complexes during *Trypanosoma cruzi* life cycle, 2) detailed characterization of the interaction between the proteins of the translation initiation machinery and the messenger RNA. By developing chemical tools and solving threedimensional structures of the protein targets and complexes at the atomic level, the teams expect to contribute to the understanding of molecular mechanisms which regulate the protein synthesis in pathogenic trypanosomatids, providing a basis for the development of selective inhibitors targeting these parasites. Chagas Disease is endemic in 21 countries of continental Latin America.

Around 7M people are infected by Chagas disease worldwide with a mortality rate over 10,000 deaths per year.


### Identification of therapeutic naturally-occurring compounds and extracts for mitochondrial diseases Project PTR n° 677- 2023 PhageSafety



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#### **PROJECT SUMMARY**



#### **KEYWORDS**

Bacteriophage, biosafety, pneumonia, chronic infection, antimicrobial, nosocomial infection, therapy, preclinical, therapy, quality control



#### ANTIMICROBIAL RESISTANCE BURDEN

Antimicrobial resistance (AMR) is on the top 10 of global health concern with significant implications for health, food security, and development (WHO). Indeed, hundreds of thousands lives are lost each year due to this resistance. In this contexte, phages were applied as an antimicrobial therapy almost immediately after their discovery in the early 20th century, to combat bacterial infections (i.e. dysentery and cholera) in humans. The lack of new antibiotics has spurred fresh interest in the use of bacteriophages (phages) to treat infections, namely phage therapy. The large variety of indications and pathogens targeted, the time-and geographical changes in infectious bacterial species and the inevitable emergence of phage resistance point to the need for tailor-made solutions. Therefore, a "personalized" approach of phage therapy is gaining weight, supported by an increasing number of positive clinical outcomes reported. However, clinical-grade phage suspensions are currently poorly characterized before their administration to patients. In particular, the immune response to phage suspensions is understudied with preliminary reports describing both positive and negative impacts on health.

#### **IMMUNOSAFETY OF PHAGE-THERAPIES**

While standardized phage cocktails show low efficacy, treating patients with tailor-made approaches of phage therapy would be better, given their antagonistic evolution and their extremely narrow host spectrum. Current phage Active Pharmaceutical Ingredients (pAPIs) are purified from bacterial lysates, and often pathogenic strains from patients are used for phage production. The quality control of pAPIs for human use entails control of their identity, purity, sterility and endotoxin levels. However, bacteria also produce non-endotoxin pyrogens, which remain undetected by the current control process but could trigger adverse immune responses in patients. As a consequence, clinical-grade phage suspensions are currently poorly characterized before their administration to patients and the immune response to phage suspensions is understudied. There is an urgent need to characterize the immunonological safety of phages in order to provide clinicians and regulatory agencies with relevant and solid scientific data that will further increase the access of phages to the patients in need.

#### THE PROJECT

The project will contribute to the effort to further characterize the interactions between phages and the immune system, in order to understand which phage to use and which phage to avoid. The teams will follow a a comprehensive characterization of both early and late immune responses to phages. Finally, they will determine whether a single, or a panel of, biomarker would better reflect the safety of pAPIs. To address this question, the project will aim to identify the most appropriate biomarker(s) that ultimately could be implemented to define new thresholds and standards specific to pAPIs. The identification of such biomarkers will require a two steps strategy. Data that will be generated from this project will certainly open new avenues to decipher how the immune system is dealing with therapeutic phages.

AMR caused 1.2 millions deaths in 2019



### Maternal effect in *Anopheles gambiae*: a proteo-transcriptomic analysis of the differential susceptibility of mosquito progeny to *Plasmodium falciparum* Project PTR n° 688- 2023 ANOPLASM



#### SCIENTIFIC COORDINATOR

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#### PASTEUR NETWORK COLLABORATORS

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#### **PROJECT SUMMARY**



#### **KEYWORDS**

Malaria, mosquito, *Anopheles gambiae*, *Plasmodium falciparum*, diseases, epigenetics, Tandem Mass Tag, vector-borne disease,



#### MALARIA:

Malaria is a life-threatening disease transmitted to humans primarily through the bites of female Anopheles mosquitoes infected with Plasmodium parasite, primarily in tropical countries. Malaria is not a disease that spreads directly from person to person. The disease is caused by a parasite that will trigger a range of symptoms, from mild to severe. Mild symptoms typically include fever, chills, and headaches. However, in severe cases, individuals may experience fatigue, confusion, seizures, and difficulty breathing. Certain groups are at a higher risk of developing severe malaria, including infants, children under the age of 5, pregnant women, travelers to malariaendemic areas, and individuals with HIV or AIDS. Malaria prevention strategies involve avoiding mosquito bites through the use of protective measures such as insect repellent and bed nets, as well as taking prophylactic medicines when traveling to regions where malaria is prevalent. Early diagnosis and treatment are crucial in managing the disease and preventing mild cases from progressing to severe, lifethreatening illnesses.

#### THE ALARMING SPREAD OF MALARIA

There are five species of Plasmodium parasites that can cause malaria in humans. Among these, Plasmodium falciparum and Plasmodium vivax pose the greatest threats. P. falciparum is known for its high fatality rate and is particularly prevalent in Africa, while P. vivax is the dominant malaria parasite in most regions outside of sub-Saharan Africa. Because of geographic expansion of resistance of Plasmodium falciparum to several drugs and notably Artemisinin, as well as increased mosquito resistance to insecticides, novel strategies to control malaria are intensively searched and specifically the ones that would suppress transmission of the parasite by mosquitoes. This is notably of huge concern with the recent invasion of Anopheles stephensi, from Asia to Africa, as this mosquito species is responsible for urban malaria. In this context, the leading team of the project demonstrated that the susceptibility of Anopheles gambiae, the major African malaria vector to P.falciparum, is maternally controlled. The results revealed that progeny from old females are more susceptible to the parasite than progeny from young females.

#### THE PROJECT

The project aims at deciphering the molecular basis of the maternal effect leading to higher susceptibility to *P.falciparum* and longer lifespan of female progeny born from old females. To achieve this aim, the teams will take advantages of a multidisciplinary approach mixing comparative RNA-seq analysis, Tandem Mass Tag (TMT), that will be combined through statistical analysis and bioinformatics. As the genotypes of mothers and progeny are similar, results from this project should open the way for understanding/identifying epigenetic signatures controlling the studied phenotypes. Overall, this novel knowledge could then be translated into innovative ways for better controlling malaria transmission acting on the mosquito vector.

247 million Malaria cases were detected in 2022

In 2021, half of the world's population was at risk of malaria.



# Role f human auto-antibodies against nicotinic acetylcholine receptors in psychiatric disease Project PTR n° 695-2023 Anti-NIC



#### SCIENTIFIC COORDINATOR

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#### **PROJECT SUMMARY**



#### **KEYWORDS**

schizophrenia, bipolar disorder, biomarker, nicotinic acetylcholine receptors, auto-antibodies, autoimmunity, memory, learning, attention, psychotic disorders, innate immunity, adaptative immunity



#### BRAIN DISEASES: COMPLICATED ORIGINS

Mental disorders are the most debilitating and costly diseases in developed countries. Due to their complexity, their heterogeneity and the absence of biomarkers, the understanding of the causes of psychotic disorders remains limited and hampers the development of new specific treatments. But there is growing recognition that the immune system (especially auto-immunity) may be a key player. Indeed, there is a well-described disease, called autoimmune encephalitis, in which the immune system attacks the nervous tissue, leading to severe psychiatric symptoms. This allows to suggest that a more comprehensive understanding of the impact of auto-antibodies (AAbs) on the nervous system would pave the way to a better understanding of the etiology of psychotic disorders.

#### NICOTINIC ACETYLCHOLINE RECEPTORS: STUDY OF THE LINK BETWEEN AAbs AND PSYCHIATRIC DISORDERS

One interesting piece of evidence to resolve the link between AAbs and psychotic symptoms could come from another disease: *Myastenia gravis*. Indeed, this auto-immune disease is caused by **AAbs targeting an** essential actor of neuromuscular junctions: the **muscle-type nicotinic Acetylcholine Receptors (nAChRs)**. This proves that AAbs targeting nAChRs can have direct consequences on human health. Importantly, there are also the **neuronal-type nAChRs**, which are widely represented in the Central Nervous System (CNS) and also found in the periphery and immune cells. Neuronal nAChRs play an important role in the regulation of pro-inflammatory cytokine release. It has also been demonstated that neuronal nAChRs are also targeted by AAbs, leading to autoimmune autonomic neuropathies.

A link between nAChRs and psychotic disorders is supported by observations from patients. Firstly, psychiatric patients often have an addiction to nicotine (activator of nAChRs); secondly, genetic mutations leading to insufficient function of neuronal nAChRs are associated with mental illnesses like schizophrenia (SZ); finally, this receptor plays a crucial role in the inflammatory response, since it is present in immune cells apart from its abundance in the nervous system. Therefore, studying how antibodies directed against these neuronal nAChRs may contribute to mental illness, could open up new avenues for the development of therapeutic approaches targeting the immune system.

#### **THE PROJECT**

This project aims to characterize AAbs targetting neuronal nicotinic receptors and to produce them for in-depth molecular, structural and functional analysis *in vitro* and *in vivo*. Exploring both clinical and mechanistic aspects of these AAbs will contribute to the multi-scale characterization of psychiatric diseases from bedside-to-bench and is expected to pave the way for innovative treatment strategies aiming to reduce these AAbs. The teams will use samples of human neuronal nAChR subunits to characterize nAChR AAbs in the blood of SZ and bipolar disorder (BD) patients and compare them to control subjects. The consortium will then study the consequences of the presence of these AAbs on brain activity and behavior *in vivo*. This project will decipher and demonstrate the role of AAbs against nAChRs in SZ and BD with the goal to elucidate their contribution to the consistently reported chronic peripheral inflammation in these patients. Moreover, the project will identify novel biomarker candidates for such diseases and should, in the long-term, help to personalize treatment approaches for patients with psychosis.

38.2% of European population is affected by a mental disorder at least once in their lifespan.



## Structure/function of a multiprotein complex binding the LXGtoxin TelE, a prototypal T7SSb effector

Project PTR n° 708- 2023 SecreT7



#### SCIENTIFIC COORDINATOR

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#### **PROJECT SUMMARY**



#### **KEYWORDS**

T7SSb, *Streptococcus gallolyticus subsp. gallolyticus*, emergence, bacteria, TelE, microbiota, gut colonization, cancer, LXG-effectors



### EMERGENT PATHOGEN KEY MECHANISM : Streptococcus

#### gallolyticus subsp. Gallolyticus (S. gallolyticus)

Streptococcus gallolyticus subsp. Gallolyticus is a Gram-positive bacteria which is an emerging cause of septicemia and endocarditis in the elderly and its presence is strongly linked to colon cancer.

The consortium teams previously demonstrated that Sgg expresses pathogenic factors that strongly enhance its ability to colonize the colon, particularly in tumoral conditions. Indeed, the consortium teams identified a genetic locus encoding a Type VIIb Secretion System (T7SSb), absent in the other subspecies of *Streptococcus gallolyticus* (macedonicus or pasteurianus). T7SSb mays be part of Sgg arsenal to defeat competitors in the host colon. A recent study indicates that the T7SS of *S.gallolyticus* strain contributes to gut colonization and tumor development, but the mechanisms are unknown.

## T7SS: A KEY ACTOR SYSTEM FOR INSTESTINE INVASION BY *S.GALLOLYTICUS*

In Firmicutes bacteria, T7SSb secretes toxic LXG-effectors for bacterial competition. The secretion and action mechanisms of toxins released through the T7SS are still not well-understood. It was first discovered in *Mycobacterium tuberculosis* in which it secretes key virulence factors required for infection. But, research on secretion systems in Gram-positive bacteria, especially focusing on their structural components, presents, so far, a significant challenge. Consequently, the prospect of investigating a seven-protein complex; here composed by TeIE, TipE and 5 other actors; within this context is groundbreaking and has the potential to advance the field significantly. It's even more important to unverstand better those mechanisms as they are key actors for *Streptococcus gallolyticus*, an emergent pathogen. Indeed, the T7SS is crucial in competing with the patient's own microbiota, enabling the pathogen to colonise the intestine and cause colorectal cancer.

#### THE PROJECT

To understand the mecanisms underlying the T7SS the teams aims to perform a structural characterization of the TelE effectors involved in its secretion and activity modulation. Using an *in vitro* gut model system, researcher will analyze TelE interaction with host's cells membrane leading to patient's gut colonization. Furthermore, the teams will unravel the *S.gallolyticus* competition potential against patient's microbiote: they will identify bacterial species specifically targeted by the *S.gallolyticus* T7SS system. Understanding how TelE is secreted and operates will provide valuable insights into the role of T7SS in bacterial competition and how S. gallolyticus manages to outcompete other microorganisms in the gut microbiota. Furthermore, this research may identify TelE as a potential therapeutic target for addressing gut-related health issues. In summary, this project holds great promise for enhancing our comprehension of bacterial warfare and its implications for human health.

60% of people infected by S.gallolyticus present cancerous lesions.



# Annexes



## List of funded Incentive Programs from 2014 to 2022

### INTER-PASTEURIAN CONCERTED ACTIONS (ACIP)

#### • 2014

-ACIP 02-2014: Bionomics, Receptivity to *Plasmodium falciparum* and Susceptibility to insecticides of *Anopheles sergentii* in the Maghreb / Zoubir Harrat - Laboratory of Parasitic Eco-Epidemiology and Populations Genetics - Institut Pasteur in Algeria

-ACIP 03-2014: «Apport du génotypage sur la surveillance des infections invasives meningogocciques : impact sur la politique vaccinal» / Muhamed-Kheir Taha - Infection and Epidemiology Department - Institut Pasteur (Paris)

-ACIP 04-2014: Evaluation of the use of non-invasive tests for early screening and survey of arboviruses (Arbo-VIRTUESS = ArboVIRus non-invasive Test Use for Early Screening and Survey) / Nancy Roosens - Platform Biotechnology and Molecular Biology - Scientific Institute of Public Health (Brussels, Belgium)

-ACIP 06-2014: Development of a standardized rat model of *Mycobacterium tuberculosis* infection to screen protective efficacy of novel TB vaccine candidates / Carine Rouanet - Center of Infection and Immunity of Lille (CIIL) - Institut Pasteur (Lille, France) \*

-ACIP 07-2014: Mediterranean Autism Project (MAP) / Thomas Bourgeron - Neuroscience Department - Institut Pasteur (Paris)

-ACIP 09-2014 : Modélisation de la transmission et du maintien des virus de la dengue en milieu urbain au Laos et au Cambodge / Malayvanh Lao - Laboratory of arboviruses and emerging viral diseases - Institut Pasteur in Laos

-ACIP 11-2014: The many faces of Hepatitis C virus: Impact of defective genomes on pathogenesis of Liver disease by assessment of exosomes secretion / Urania Georgopoulou - Microbiology Department - Hellenic Pasteur Institute (Greece) \*

-ACIP 14-2014: Role of intestinal carriage in the global emergence of multidrug resistant and hypervirulent clones of *Klebsiella pneumoniae*: a population biology approach / Sylvain Brisse - Genomes and Genetics Department – Institut Pasteur (Paris) \*

-ACIP 15-2014: Diagnostic, évolution moléculaire et compétence vectorielle pour *Aedes aegypti* du virus Zika en Afrique, Asie et dans le Pacifique / Myrielle Dupont-Rouzeyrol - Dengue and Arboviruses Expertise and Research Unit - Institut Pasteur in New Caledonia and Oumar Faye - Arbovirus and Viral Hemorrhagic Fever Unit - Institut Pasteur in Dakar

#### • 2015

-ACIP 03-2015: Hepatitis E in Cambodia and Vietnam: Is it emerging? / François Rouet - HIV / Hepatitis Unit - Institut Pasteur in Cambodia

-ACIP 10-2015: Circulating biomarkers in geographically diverse populations with varying rates of gastric cancer: mitochondrial DNA and infammatory factors as potential candidates / Eliette Touati - Microbiology Department - Institut Pasteur (Paris)

-ACIP 13-2015: The impact of micro-RNAs and infammatory pathways on stem cell fate and the regenerative process in human inherited muscle diseases / Shahragim Tajbakhsh - Developmental and Stem Cell Biology Department - Institut Pasteur (Paris)

-ACIP 17-2015: Target-based drug discovery of compounds interfering with trypanothione biosynthesis in trypanosomatids / Marcelo Comini - Laboratory Redox Biology of Trypanosomes - Institut Pasteur in Montevideo

-ACIP 18-2015: Identification of broad-spectrum naturally derived inhibitors against hepatotropic viruses (DENV, YFV, HBV) under culture conditions simulating liver normoxic and metabolic microenvironment. The interplay between virus and hepatic normoxia as a possible disease determinant / Niki Vassilaki – Molecular Virology Laboratory - Hellenic Pasteur Institute (Athens, Greece)

-ACIP 22-2015: Insecticide resistance of Aedes aegypti and Aedes albopictus from Central African Republic, Madagascar and French Guiana / Carine Ngoagouni - Virology and Medical Entomology Service - Institut Pasteur in Bangui

#### • 2016

-ACIP 01-2016: Are Ae. aegypti from "clean" breeding sites more dangerous than those from "dirty" ones?: Infuence of breeding sites bacterial communities on Aedes aegypti microbiome, survival and vector competence (AEGYPTERIA) / Anubis Vega Rua - Laboratory of Medical Entomology - Institut Pasteur in Guadeloupe

-ACIP 03-2016: Development of a novel, ultra-fast diagnostic platform for identification and drug-susceptibility testing of *Mycobacterium tuberculosis* (TIDRACE / Tuberculosis Identification and Drug Resistance by AbsenCe of Expression) / An Van den Bossche - Division of Bacterial Diseases - Scientific Institute of Public Health (Brussels, Belgium) \*

-ACIP 06-2016: Dengue virus genotype replacements: investigating viral fitness differences driving the evolution of dengue epidemics (DenGen) / Myrielle Dupont-Rouzeyrol -Dengue and Arboviruses Expertise and Research Unit - Institut Pasteur in New Caledonia

-ACIP 22-2016: Viral etiology and clinical features of Hand, Foot and Mouth disease in Central African Republic and Senegal (GENTERO) / Abdou Kader Ndiaye - Enteric Viruses Laboratory - Institut Pasteur in Dakar

#### • 2017

-ACIP 05-2017: Animal venom biomolecules: Tools for oncogenesis study and targeted therapy against Hepatocellular carcinoma (HCC) (Venoms in ACIP (Anti-Cancer Innovant Polytherapy)) / Najet Srairi Abid - Laboratory of venoms and therapeutic biomolecules - Institut Pasteur in Tunis \*

-ACIP 41-2017: EXploring the hidden GENomic diversity of *Anopheles gambiae* and *A. coluzzii* species pair to account for spatial differences in MALaria transmission (ExGenMal) / Beniamino Caputo - Parasitology Unit - Institut Pasteur - Cenci Bolognetti Foundation (Rome, Italy) \*

-ACIP 45-2017: Identification of biomarkers for Type 2 Diabetes through multidisciplinary investigations (DiaBiomark) / Rym Kefi - Laboratory of Biomedical Genomics and Oncogenetics - Institut Pasteur in Tunis

-ACIP 57-2017: Microbiota-Epigenomic Crosstalk in Terminal Liver Diseases of Patients from MENA (MECrosLiv) / Pascal Pineau - Cell biology and Infection Department - Institut Pasteur (Paris)

-ACIP 76-2017: Systematic identification and functional analysis of novel centrosomal components in *Toxoplasma gondii* (Centrosome dissection of *T. gondii*) / Maria Francia - Molecular Biology Unit - Institut Pasteur in Montevideo

-ACIP 93-2017: Synthesis and biological evaluation of the inhibitors of *Plasmodium falciparum* lactate dehydrogenase enzyme (pfLDH) selected by docking studies (PlasmoLdHit) / Azar Tahghighi - Medicinal Chemistry Unit - Institut Pasteur in Iran

#### • 2019

-ACIP 162-2019: Animal Enteroviruses in Central Africa, from field to assay tubes: search for zoonotic enterovirus transmission and study of genetic exchanges between animal and human enteroviruses (AnEiCA) / Maël Bessaud - Viral Populations and Pathogenesis Unit - Institut Pasteur (Paris)

-ACIP 221-2019: Dengue virus-mosquito-host interactions: assessing type I interferon mediated immune processes and immunological effects of mosquito salivary glands on Dengue infection (DeSiReS: Dengue Severe Immune Response Studies) / Caroline Scagnolari - Laboratory of Virology - Institut Pasteur - Cenci Bolognetti Foundation (Rome, Italy)

-ACIP 253-2019: Engineering Asaia to induce RNA interference in larval and adult Anopheles: a tool for vector genetics and paratransgenesis (paratransgenesis based on RNAi and Asaia bacterium in Anopheles) / Abbasali Raz - Malaria and Vector Research Group - Institut Pasteur in Iran

-ACIP 255-2019: Ethomics for mosquitoes (ETHOMOS) / Jean-Bernard Duchemin - Medical Entomology Unit - Institut Pasteur in French Guiana

-ACIP 275-2019: Enterovirus 68 investigation in children with acute respiratory infection and acute faccid myelitis in two Sub Saharan African countries (SURVEV68) / Ndongo Dia - Respiratory Viruses Research Group - Institut Pasteur in Dakar

-ACIP 281-2019: Determinants of the expansion of dengue minority serotypes (E-DYNAMICS) / Myrielle Dupont-Rouzeyrol and Catherine Inizan - Dengue and Arboviruses Expertise and Research Unit - Institut Pasteur in New Caledonia

#### • 2020

-ACIP 318-2020: Targeting hepatitis B virus cccDNA by cellular nucleases: towards viral elimination of chronic hepatitis B (DEHBV) / Jean-Pierre Vartanian - Molecular Retrovirology Unit - Institut Pasteur (Paris)

-ACIP 328-2020: The lung epitranscriptome and chromatin accessibility landscape in rheumatoid arthritis complicated by the fu (m6A\_RAIAV) / Milena Leseva - Laboratory of Experimental Immunotherapy - Stephan Angeloff Institute (Bulgaria)

-ACIP 358-2020: Evolution of regulatory interactions through the lens of antibiotic selective pressure (EVoSP) / Teca Galvao - Laboratôrio de Genômica Funcional e Bioinformatica - Fiocruz (Brazil)

-ACIP 363-2020: Investigating the fea vectors towards understanding human bubonic plague persistence in Madagascar (Flea Vec) / Mireille Harimalala - Medical Entomology Unit - Institut Pasteur in Madagascar

-ACIP 399-2020: Ascorbate-deficiency: a novel diarrhea risk-factor? (VITAL) / Muriel Vray - Emerging Diseases Epidemiology Unit - Institut Pasteur (Paris)

-ACIP 403-2020: Antibody persistence after abridged intradermal rabies PEP: The RESIST-3 study (RESIST-3) / Arnaud Tarantola and Perrine Parize - Lyssavirus, Epidemiology and Neuropathology Unit - Institut Pasteur (Paris)

#### • 2021

#### - Identification of the rapeutic naturally-occurring compounds and extracts for mitochondrial diseases ACIP $n^\circ\!443$ - 2021

Keywords: mitochondrial diseases, genetic diseases, drug screening, microalgae, library

## - Assessment of prevalence of Leptospira spp and interaction between environment, animals and human leptospirosis in periurban areas in Africa

ACIP n°445 - 2021

Keywords: livestock, epidemiology, rodents, mapping, One Health, Leptospira interrogans

#### - Myosins-Usher2 proteins Complexes in hearing Loss

ACIP n°485 - 2021

Keywords: Ears, genetic disease, Usher2 syndrome, cilia, myosins, ADGRV1, PDZD7, whirlin, biophysics, X-ray crystallography, Nuclear Magnetic Resonance,

#### - Avian arbovirus testing, transmission analysis, and risk mapping

ACIP n°489 - 2021

Keywords: arbovirus, avian, surveillance, diagnostic tool, serosurvey, reservoir, vector, modeling, simulation, biomarkers, mapping

#### • 2022

## - Association of SARS-CoV-2 variants of concern and COVID-19 severity with the activity of catecholamine biosynthetic

#### ACIP n° 505 - 2022

Keywords: SARS-CoV-2, virus, catecholamine, L-Dopa decarboxylase, hypoxia, HIF signaling, ROS, drug screening, host pathogen interaction, antimicrobial resistance

### - Intestinal organoid models for studying host-parasite interaction in Chagas Disease

ACIP n° 532 - 2022

Keywords: Chagas disease, Trypanosoma cruzi, parasite, host-parasite, gastrointestinal tract, organoid, mini-gut, macrophage, reservoir, drug resistance,

## - Clinical and parasitological features of cutaneous leishmaniasis with treatment failure/relapse in endemic foci of Algeria and Tunisia: focus on oxidative stress markers and immune response

ACIP n° 562 - 2022

Keywords: Antimony sensitivity, cutaneous leishmaniasis, cytokine, oxidative stress, Nrf2/Keap1, parasite, treatment resistance, macrophage, Leishmania major

## - Geographic origin of the parasite Angiostrongylus cantonensis and role of the mollusc Achatina fulica in its dispersion, in Central and West Africa and in the French departments of America

ACIP n° 569 - 2022

Keywords: Anthropozoonosis, Angiostrongylus cantonensis, Achatica fulica, cytB, cytC, rodents, international, global phylogeny, mitochondrial 16S rRNA

## - Exploiting Signal-regulatory protein alpha Exploiting Signal-regulatory protein alpha (SIRPA) signaling to control virus-mediated inflammation

ACIP n° 570 - 2022

Keywords: innate immunity, inflammation, host-pathogen interactions, dengue virus, Ebola virus, hemorrhagic fever, SARS-CoV-2, Zika virus, SIRPA, TLR, macrophage

### - Characterization of the Uruguayan population using low-pass sequencing

#### ACIP n° 577 - 2022

Keywords: Genetic of population, low pass sequencing, LPS, human trait, ancestry, representation, algorithm, population characterization, variant traits

### TRANSVERSAL RESEARCH PROGRAMS (PTR)

#### • 2014

**-PTR 481-2014:** Pathogen discovery in highly selected samples from pediatric cases of acute encephalitis syndrome of unknown etiology in Southeast Asia / Marc Eloit - Cell Biology and Infection Department - Institut Pasteur (Paris) \*

**-PTR 483-2014:** The PDZ mediated interactome in the auditory sensory cells / Nicolas Wolff - Structural Biology and Chemistry Department - Institut Pasteur (Paris)

-PTR 484-2014: Human Enterovirus A71 circulation and epidemic risk in Africa: Characterization and pathogenicity of new viral genogroups / Francis Delpeyroux - Virology Department - Institut Pasteur (Paris)

**-PTR 489-2014:** Mosquito and human driven evolution of Chikungunya virus in the Americas: identifying future adaptative intermediates / Marco Vignuzzi – Virology Department – Institut Pasteur (Paris)\*

**-PTR 490-2014**: Analysis of receptor-ligand interactions involved in host cell invasion by *Plasmodium vivax* merozoites: Building the rationale for a blood stage malaria vaccine / Chetan Chitnis - Parasites and Insect Vectors Department - Institut Pasteur (Paris)

**-PTR 491-2014:** Role of peripheral blood mononuclear cells in dengue virus transmission to mosquito vectors / Marc Grandadam – Arbovirus and emerging viral diseases Laboratory - Institut Pasteur in Laos

-PTR 494-2014: Unraveling how gastric *Helicobacter* species evolved to colonize their hosts / Daniel Vinella - Microbiology Department - Institut Pasteur (Paris)

**-PTR 496-2014:** Investigating the reciprocal relationship between macrophage infammasome activity and intracellular *Leishmania* infection / Eric Prina - Parasites and Insect Vectors Department - Institut Pasteur (Paris)

-PTR 499-2014: "Deciphering the innate immune defense of New World bat species to viral infection" / Vincent Lacoste - Laboratory of Virus-Host Interactions - Institut Pasteur in French Guiana

**-PTR 505-2014:** Establishment of a reverse genetics system for hantaviruses and its use for surveillance in Madagascar wildlife / Noël Tordo – Virology department - Institut Pasteur (Paris)

**-PTR 510-2014**: Towards novel approaches for the identification of *Bacillus cereus* species and their specific characteristics (IdBc)/ Dominique Clermont - Biological Resource Center of the Institut Pasteur - Institut Pasteur (Paris)

#### • 2015

**-PTR 521-2015**: Identification of microRNAs regulating infection of human cells by *Listeria monocytogenes* / Javier Pizarro- Cerda – Cell Biology and Infection Department – Institut Pasteur (Paris)

**-PTR 523-2015**: Mechanisms of pathological A53T-alpha-synuclein transmission in human iPS-derived neurons / Rebecca Matsas - Laboratory of Cellular and Molecular Neurobiology - Hellenic Pasteur Institute (Athens, Greece)

**-PTR 528-2015**: Risk of reemergence of urban yellow fever in Brazil: role of the invasive mosquito *Aedes albopictus /* Ricardo Lourenço-de-Oliveira - Laboratorio de Transmissores de Hematozoarios - Fiocruz (Brazil)

**-PTR 529-2015**: Aspergillus fumigatus Hydrophobins: Role in conidial/mycelial structure and their implication on virulence / Anne Beauvais - Mycology Department - Institut Pasteur (Paris)

**-PTR 535 -2015**: Beyond Artemisinin Resistance Associated to *Plasmodium* KI3: towards insights on KI3 biological function (Be-ART<sup>R</sup>) / Jean-Christophe Barale - Structural Biology and Chemistry Department - Institut Pasteur (Paris)

**-PTR 539-2015**: A multilevel systems approach to elucidate the host-*Leishmania* interactome and to identify host targets for anti-leishmanial drug discovery / Joo Hwan No - Leishmania Research Laboratory - Institut Pasteur Korea

**-PTR 540-2015**: Beyond acid resistance: Role of bacterial *y*-aminobutyrate (GABA) in community and host interactions (GABActeria) / Jean-Marc Ghigo – Microbiology Department – Institut Pasteur (Paris)

-PTR 542 – 2015: Deciphering the impact of *Plasmodium/Trypanosoma* co-infections on the vectorial capacity of *Anopheles* mosquitoes / Christian Mitri - Parasites and Insect Vectors Department - Institut Pasteur (Paris)

**-PTR 546-2015**: Deciphering the infuenza viral polymerase interplay with host ubiquitin-proteasome system in correlation with pathogenesis / Caroline Demeret – Virology Department – Institut Pasteur (Paris)

-PTR 558-2015: Acquisition of antibiotic resistant bacteria and development of the gut microbiome in neonates in low-income countries / Philippe Glaser - Microbiology Department - Institut Pasteur (Paris)

#### • 2016

**-PTR 08-2016**: Mucosal and systemic immune responses in children suffering from malnutrition and environmental enteropathy (Immunohealth) / Philippe Sansonetti and Pascale Vonasch - Cell Biology and Infection Department - Institut Pasteur (Paris)

**-PTR 10-2016**: Role of PDZ-binding motif from Flavivirus non-structural proteins in faviviral life cycle (FlaviZome) / Nathalie Pardigon - Infection and Epidemiology Department - Institut Pasteur (Paris)

-PTR 18-2016: Role of Nod1 in host/bacteria interactions in allergic asthma (NODASTHMA) / Anne Tsicopoulos - Center for Infection and Immunity - Institut Pasteur (Lille, France)

**-PTR 20-2016**: Complexity of immune interactions in chronic hepatitis B virus infection: how the exacerbated infammatory response by self-antigen specific CD8 T cells and regulatory T cells dictate the fate of HBV specific responses (SELF B-Liver) / Maryline Bourgine - Virology Department - Institut Pasteur (Paris)

**-PTR 22-2016**: Reconstitution of *Mycobacterium* and *Shigella* infection in physiological condition using organ-on-achip (Shig&Tub-on-a-Chip) / Nathalie Sauvonnet - Cell Biology and Infection Department - Institut Pasteur (Paris)

**-PTR 24-2016**: Understanding the selective benefit of the *Shigella* genome specific architecture (SHIGENARCH) / Bianca Colonna - Laboratory of Molecular Microbiology - Institut Pasteur - Cenci Bolognetti Foundation (Rome, Italy)

-PTR 26-2016: Aspergillus and Aspergillosis in Cambodia (AAC) / Jean-Paul Latgé - Mycology Department - Institut Pasteur (Paris)

-PTR 35-2016: The Healthy Human Global Project - Senegal / Pilot study to characterize the variability of the immune

response in two Senegalese populations (HHGP-Senegal) / Aissatou Touré - Immunology Unit - Institut Pasteur Dakar and Darragh Duffy - Immunology Department - Institut Pasteur (Paris)

**-PTR 39-2016**: A multi-disciplinary investigation of the *Negativicutes*: Atypical *Firmicutes* with LPS-outer membranes that inhabit the human gut (OMNEGA) / Simonetta Gribaldo - Microbiology Department - Institut Pasteur (Paris)

-PTR 43-2016: The Role of ExoY nucleotidyl cyclase toxin in *Pseudomonas aeruginosa* infections (ExoY) / Undine Mechold - Structural Biology and Chemistry Department - Institut Pasteur (Paris)

#### • 2017

**-PTR 03-2017**: Toxins and nanobodies as subtype-specific ligands and allosteric modulators of nicotinic acetylcholine receptors (Nicobinder) / Pierre-Jean Corringer - Neuroscience Department - Institut Pasteur (Paris)

-PTR 24-2017: Role of HP1/Cbx protein ubiquitination in chromatin organization (HPUCO) / Giovanni Cenci - Institut Pasteur - Cenci Bolognetti Foundation (Rome, Italy)

-PTR 30-2017: Global diversity, genomic epidemiology and pathogen evolution of *Leptospira* (GlobE Evolution) / Mathieu Picardeau - Microbiology Department - Institut Pasteur (Paris)

-PTR 52-2017: Anti-tuberculosis vaccination using new generation live attenuated vaccines and lentiviral vectors encoding stage-specific mycobacterial antigens: prophylactic and therapeutic approaches (TB-LAV/LV-VAC) / Marta Romano - *In vivo* models unit / Immune Response Service - Scientific Institute of Public Health (Brussels, Belgium)

**-PTR 66-2017**: Role of Toll-Like and Nod-Like Receptors in the activation and functions of phagocytes infected with pathogenic *Leptospira* (LEPTOPHAGO) / Catherine Werts - Microbiology Department - Institut Pasteur (Paris)

**-PTR 73-2017**: Structure, function and evolution of transport systems of nickel, an essential virulence determinant of the bacterial pathogen *Helicobacter pylori* (TransNickel) / Charles Calmettes - INRS - Institut Armand Frappier (Canada)

**-PTR 91-2017**: Investigation of the possible transmission of a dysbiotic microbiota from mothers to their offspring in the context of chronic malnutrition and environmental enteropathy (Mitica) / Philippe Sansonetti and Violeta Moya-Alvarez - Molecular Cell Biology and Infection Department - Institut Pasteur (Paris)

**-PTR 98-2017**: High throughput selection of nanobodies by droplet based microfuidics (NanoFluidic) / Pierre Lafaye – Structural Biology and Chemistry Department - Institut Pasteur (Paris)

**-PTR 111-2017**: Mechanisms driving normal and accelerated ageing (RejuvenAge) / Shahragim Tajbakhsh - Developmental and Stem Cell Biology Department - Institut Pasteur (Paris)

-PTR 113-2017: Dissecting roles for diverse innate lymphoid cell subsets in colitis-induced colorectal cancer (ILC-INF-CRC) / Angela Santoni - Molecular Immunology and Immunopathology Unit - Institut Pasteur - Cenci Bolognetti Foundation (Rome, Italy)

#### • 2019

**-PTR 161-2019**: Epidemiology and Genetic Diversity of Enteroviruses in Central Africa and in Madagascar: uncovering the hidden parts of the enterovirus ecosystems (EVinCA&M) / Maël Bessaud - Viral Populations and Pathogenesis Unit - Institut Pasteur (Paris)

-PTR 166-2019: Inhibitor Screening, Cryo-Electron Microscopy, X-ray studies of CyaA (InSCEMiX) / Alexandre Chenal - Biochemistry of Macromolecular Interactions Unit - Institut Pasteur (Paris)

**-PTR 175-2019**: Development of a field-adapted CRISPR-based method for the diagnosis of sleeping sickness (SHERLOCK4HAT) / Brice Rotureau - Trypanosome Transmission Group - Institut Pasteur (Paris)

-PTR 183-2019: Transcriptional Response for AntimiCrobial Resistance detection in TB (TRACeR-TB) / An Van den Bossche - Bacterial Diseases Unit - Sciensano (Belgium)

**-PTR 190-2019**: Molecular and structural tools for deciphering trypanosomatid-specific features of EIF4E in the translational machinery (CaptoTryp) / Beatriz Guimares - Structural Biology and Protein Engineering Unit - FIOCRUZ (Brazil)

**-PTR 212-2019**: Towards new vaccine strategies for dengue virus infection: identification of protective humoral immune responses in asymptomatic dengue-infected individuals (ASDENV) / Tineke Cantaert - Immunology Unit - Institut Pasteur in Cambodia

**-PTR 218-2019**: A One Health study of monkeypox: human infection, animal reservoir, disease ecology and diagnostic tools (AFRIPOX) / Emmanuel Nakouné - Arbovirus, emerging viruses and zoonosis Unit - Institut Pasteur in Bangui<sup>2</sup>

**-PTR 232-2019**: Computational imaging of the spatiotemporal distribution of forces in gut tissue: a study of the cross talk between cell mechanics, microbiome and infectious processes (MECHABIOME) / Elisabeth Labruyere - Bioimage Analysis Unit - Institut Pasteur (Paris)

**-PTR 233-2019**: Targeting Malaria Epigenetics (TaME) / Ludovic Halby - Epigenetic Chemical Biology Unit - Institut Pasteur (Paris)

**-PTR 237-2019**: Field Tests for Rabies Diagnostic (FiTeRaD) / Laurent Dacheux - Lyssavirus Epidemiology and Neuropathology Unit - Institut Pasteur (Paris) <sup>1</sup>

**-PTR 245-2019**: Understanding RavA-ViaA sensitization to aminoglycosides and how it can be harnessed to fight antibiotic resistance (RAVVIA) / Zeynep Baharoglu - Bacterial Genome Plasticity Unit - Institut Pasteur (Paris)

**-PTR 272-2019**: Organoids-on-chip: role of Sonic-Hedgehog signaling on the development of inner ear organoids (OrganoEar) / Raphael Etournay - Genetics and Physiology of Hearing Unit - Institut Pasteur (Paris)

#### • 2020

**-PTR 291-2020:** Functional consequences of pathogen infection in tissue specific stem cells (MusVir) / Barbara Gayraud- Morel - Stem Cells and Development Unit - Institut Pasteur (Paris)

**-PTR 303-2020:** Neonatal acquisition of ESBL-PE in the community of a low-income country (Neo-LIC) / Bich-Tram Huynh - Epidemiology and modelling of bacterial escape to antimicrobials Unit - Institut Pasteur (Paris)

**-PTR 310-2020:** Adaptation of pathogenic *Leptospira* to oxidative stress: its contribution to virulence and host colonization (LEPTOXINFECTION) / Nadia Benaroudj - Biology of Spirochetes Unit - Institut Pasteur (Paris)

**-PTR 332-2020:** *E. coli* and *H. pylori* as models for the role of DNA methylation in the relation between bacteria and cancer (CoPyMe) / Eliette Touati - Unit of Helicobacter Pathogenesis - Institut Pasteur (Paris)

**-PTR 337-2020:** Validation of *Plasmodium falciparum* antigens targeted by human CD8+ T cells using T Cell Receptorexpressing reporter cells (PfAVAL) / Rogerio Amino - Malaria Infection and Immunity Unit - Institut Pasteur (Paris)

**-PTR 353-2020:** Host pathogen interactions in humans infected with zoonotic foamy viruses (ZOOFOAMENV) / Florence Buseyne - Epidemiology Physiopathology Oncogenic Viruses Unit - Institut Pasteur (Paris)

**-PTR 377-2020:** Microglia imaging in Alzheimer's disease (MIAD) / Marcos Costa - Molecular determinants of neurodegenerative diseases Unit - Institut Pasteur (Lille, France)

**-PTR 380-2020:** Development of heptose-containing semi-synthetic glycoconjugate vaccines against campylobacteriosis (CAMPYVAC) / Charles Gauthier - Centre Armand Frappier Santé Biotechnologie, INRS (Canada)

**-PTR 388-2020:** Refining the role of nicotinic acetylcholine receptors and their relevance as therapeutic targets in neuropsychiatric disorder endophenotypes (NICOGNIBRAIN) / Morgane Besson - Integrative neurobiology of cholinergic systems Unit - Institut Pasteur (Paris)

**-PTR 395-2020:** Structural and functional characterization of a Dot1-like methyltransferase encoded by *Legionella pneumophila*: bacteria-induced epigenetics? (LegiDOT) / Monica Rolando - Biology of Intracellular Bacteria Unit - Institut Pasteur (Paris)

1: co-funding Institut Pasteur - Institut Carnot Pasteur MS

<sup>2</sup>: co-funding Institut Pasteur - ANR

\*: The technical sheet of this project is currently not available (licensed to a third party / confidential information / writing in progress / involving no partner in Paris)

#### • 2021

## - Exploring Host factors linked to phagosomal rupture by Mycobacterium tuberculosis using High Content phenotypic Screening and Lung-on-Chip techniques

PTR n°430 - 2021

Keywords: Mycobacterium tuberculosis, Mtb, organ on a chip, lung, ex vivo, anti- microbial resistance, siRNA screening, FRET, IPSC, LoC, host-pathogen interaction

## - Tuning the ADAR1 RNA editing enzyme to boost type I IFN and NK cell innate immune responses in the model of HPV transformed cells

#### PTR n°432 - 2021

Keywords: Human papilloma viruses (HPVs), epithelial cells, immune response, ADAR, cell model, 3D bioprinting, cervical cancer, immune system, interferon (IFN)

## - Spatially resolved proteomics and morphodynamics of hematopoeietic stem cell aortic precursors PTR $n^\circ\,439$ - 2021

Keywords: Zebrafish, blood cancer, stem cell, immunity, hematopoiesis, HSC, embryogenesis, regenerative medicine, bona fide, ArhGEF11, APEX2, bone marrow

## - Proof of concept study: targeting DENV NS1 to reduce viremia and alleviate plasma leakage PTR n° 448 - 2021

Keywords: arbovirus, flavivirus, Dengue, DENV, NS1, high-throughput, high-content, screening, chemical, BRET, infection assay, preclinical, mouse model, antivirals.

## - High-Resolution virome of mosquitoes coupled to the quest for novel human arboviruses in Madagascar PTR n°458 - 2021

Keywords: Arbovirus, mosquitoes, arthropod, emergence, outbreak, epidemic, diagnostic, screening, LIP, seroprevalence, NGS, virome, Madagascar

#### - Tackling a Global Emerging Threat: Multidrug-Resistant Yeast Candida auris

PTR n° 459 - 2021 Keywords: Candidiasis, Candida auris, multi-drug resistance, fungi, high throughout, drug screening, kinase, mouse model, inducible expression

#### - Centrosome and basal body function in human parasites

PTR n°474 - 2021 Keywords: Parasite, apicomplexa, Trypanosoma brucei, Toxoplasmosa gondii, flagella, centriole, basal body, phylogeny, RNAi, protein structure

• 2022

## - Optimizing CyaA translocation for its use in biotechnological applications as enhanced antigen delivery vehicle PTR n°502 - 2022

Keywords: CyaA, Bordetella pertussis, whooping cough, ACD, CaM, P454, ADV, immunization, vaccine, antigen delivery vehicle, AI, protein confinement

#### - Effect of mood states on olfactory system neural connectome

PTR n° 511 - 2022

Keywords: Depression, olfaction, Neural architecture, microscopy, machine learning, Virtual Reality

#### - Neuronal excitability as a neurophysiological substrate for sensitivity to sounds

PTR n° 520 - 2022

Keywords: Diagnosis, Biomarkers, model for research, autism, hyperacusis, models, sensory disorder, sound roughness,

anxiety, hyperresponsiveness, hyperexcitability

#### - Targeting the peptidoglycan assembly complexes

PTR n° 526 - 2022

Keywords: nucleic acids, vaccines, antimicrobial, PGN, gastric ulcer, Helicobacter pylori, bacteria, cell envelope, antibiotic resistance, Penicillin-binding protein, MreC

#### - Mechanisms of Heteroresistance leading to antifungal treatment failure

PTR n° 530 - 2022

Keywords: mycology, fungal pathogens, Candida albicans, candidiasis, antifungal resistance, azole heteroresistance, microfluidics, population heterogeneity

#### - Mechanics of Inviding Glioblastoma Cells

PTR 548 - 2022

Keywords: Glioblastoma cells, glial cells, cancer, cell junction, cell-adhesion, 3D cell culture, microscopy, cell migration, cell invasion, intermediate filaments, cytoskeleton

#### - Comprehensive profiling of human and mouse metastasis-infiltrating ILC

PTR n° 550 - 2022

Keywords: Transcriptomics, Innate immunity, tumour, immunology, liver, lung, ILC1, NK cell, cancer, metastasis, single cell, RNA-seq, cell-sorting

#### - Targeting mitochondrial membrane integrity in Chagas Disease

PTR n° 558 - 2022

Keywords: Chagas disease, Trypanosoma cruzi, mitochondria, metabolism, parasite, immunity, mice model, organelle, cell model, cardiomyopathy, preclinical

#### Integrative approach to combat antibiotic resistance by targeting protein- protein interactions essential to bacterial macromolecular machinery.

PTR n° 566 - 2022

Keywords: Antimicrobial resistance, antibiotic, therapeutical strategy, divisome, Fr- PPI-Chem Library, Bacterial Two Hybrid Screening Technology, BACTH, E. Coli

#### - APOBEC3CI188 mutator enzyme, a genetic predisposition marker for liver cancer

PTR n° 588 - 2022

Keywords: liver cancer, A3CI188, cytidine deaminase, reverse genetics, genome editing, recombination, diagnosis, biomarker, Hepatocellular carcinoma, mouse model

## - Origins and dynamics of oral dysbiosis associated with enteric dysfunction. Preventive intervention in Central African infants.

PTR n° 596 - 2022

Keywords: Metagenomics, epidemiology, surveillance, environmental enteric dysfunction

## PORTFOLIO INTER-PASTEURIAN CONCERTED ACTIONS (ACIP) FUNDED FROM 2014 TO 2020

III

# Knowing better the malaria vector will improving its surveillance



#### Project ACIP n° 02-2014

**Coordinator:** Dr Zoubir HARRAT, Laboratory of Parasitic Eco-Epidemiology and Populations Genetics - Institut Pasteur in Algeria

**Institut Pasteur International Network collaborators:** Dr Karim AOUN, Institut Pasteur in Tunis, Dr M'hammed SARIH, Institut Pasteur in Morocco and Dr Catherine BOURGOUIN, Institut Pasteur (Paris)

#### **MALARIA**

Malaria is a potentially fatal parasitic disease caused by several species of parasites of the genus *Plasmodium*. *Plasmodium falciparum* (*P. falciparum*) affects developing resource-poor countries, mostly in sub-Saharan Africa. It is responsible for most of the mortality associated malaria.

Malaria is transmitted to humans by bites of infected female *Anopheles* mosquitoes. There are more than 400 different species of *Anopheles* mosquito; around 30 are malaria vectors of major importance. *Anopheles gambiae* is one of the best known, because of its predominant role in the transmission of the most dangerous malaria parasite species (to humans) – *P. falciparum*.

The main way to prevent and reduce the transmission of malaria is vector control (spraying insecticides inside houses, insecticide treated bed nets). Nevertheless, the control of the vector is hampered by the emergence of mosquito resistance to insecticides.

#### **MALARIA IN MAGHREB**

Major malaria eradication programs were launched in the Maghreb countries in the 60s and 70s.

In recent years, incidence of malaria has increased in these countries. The resurgence of malaria in Maghreb could result from the weakening of control programs, increased migration of human populations and/or mosquitoes behaviour changes resulting from climatic and environmental modifications.

#### **THE PROJECT**

The Institut Pasteur teams of Maghreb as well as a team of Paris propose to focus on the main North African vector, the *Anopheles sergentii* mosquito. Specifically, this project will enable researchers to obtain accurate and update information on the ecology of this main vector (in Tunisia, Morocco and Algeria), its role in the spread of the malaria in these countries as well as its susceptibility to insecticides. With this knowledge, an epidemiological surveillance and alternatives in malaria control plans will be organized in order to prevent outbreaks. International budget for the control and elimination of malaria in 2013: 2,7 billion of dollars.

Morocco was declared malariafree in 2010 but lately 100 cases per year have been identified.

### Enhancement fo the epidemiological surveillance of Invasive Meningococcal Infections for implementation of vaccine strategies



#### Project ACIP n° 03-2014

Scientific coordinator: Dr Muhamed-Kheir TAHA, Invasive bacterial infections Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Aziza RAZKI, Institut Pasteur in Morocco and Dr Hassiba TALI-MAAMAR, Institut Pasteur in Algeria \*

#### WHAT IS NEISSERIA MENINGITIDIS?

*Neisseria meningitidis* (N.m) is a Gram-negative bacteria of the normal nonpathogenic flora in the nasopharynx in 10% of the general population. It colonizes and infects only humans, and is the main cause of Invasive Meningococcal Infections (IMI) in children or young adults.

*N.m* spreads during interhuman contacts through saliva and respiratory secretions of infected persons (including asymptomatic carriers) during coughing, sneezing, kissing and chewing on toy. The transmission rate is higher in closed and semiclosed environments (military recruits, university students, family contacts).

Currently, 12 serogroups have been described of which 6 (A, B, C, W, Y and X) are responsible for almost all IMI.

IMI are notifiable diseases and represent a major health problem in the world. IMI are often sporadic in developed countries and are responsible for epidemics mainly in the "meningits belt" of the subsaharan Africa.

The period incubation is short, from 2 to 10 days. The symptoms of meningitis are fatigue, fever, headache and can rapidly progress to neck stiffness, coma leading in some cases to the death of the patient or leaving significant sequelae (eg. mental retardation, deafness, epilepsy or other neurological conditions). The patient with suscepted IMI should be hospitalized immediately for treatment with antibiotic. Anyone who has had close contact with an infected patient should also receive preventive antibiotic therapy to prevent the spread of the infection.

Vaccines targeting serogroups A, C, W, Y and B are available.

#### SURVEILLANCE OF INVASIVE MENINGOCOCCAL INFECTIONS

*Neisseria meningitidis* is a highly variable bacteria due to its natural competence for transformation (frequent genetic exchange and recombination between strains). This large genetic variability allows the bacteria to modify their transmissibility and/ or invasiveness as well as to escape the immune pressure from its host, causing increases in the incidence of the disease in some parts of the world. In addition, these new variants, continuously generated, may be variants that escape the vaccines.

Furthermore, the displacement of communities and the current socio-economic changes are not without consequences on the modifications of the strains in circulation. With the displacement of the communities or migratory flow, cross-border epidemics affecting several countries must be identified in order to react in a coordinated manner.

Bacteriological surveillance is therefore crucial i) for the earliest possible alert to control the risk of an epidemic ii) to establish an optimal vaccine strategy based on circulating strains in each region iii) to detect the appearance of new variants and adapt vaccine strategies.

This surveillance involves the implementation of epidemiologic studies and typing using genetic and phenotypic approaches.

Invasive meningococcal disease is considered to be one of the leading causes of death: 20 to 30% worldwide during an epidemic.

The incidence of IMI varies in the world between 1 to 1,000 cases per 100,000 inhabitants per year.

In sub-Saharan Africa, 1000 new cases per 100,000 people during an epidemic.

In France, between 0.9 and 1.6 cases per 100,000 inhabitants over the last 10 years.

#### **THE PROJECT**

The objective of this project, which associates 3 teams from Pasteur Institutes of Paris, Morocco and Algeria, is the surveillance of meningococcus thanks to genetic and phenotypic approaches aiming to analyze the impact of the diversity of the strains on the epidemiology of IMI and coverage of strains by currently available vaccines.

Meningococcal isolates from IMI patient samples (from hospitals in Morocco and Algeria) will be characterized genetically to determine the genotype. An antibiotic susceptibility study will be conducted to monitor antibiotic resistance and therefore to better adapt the treatment. Then a molecular typing of the bacterial strains by the MLST (MultiLocus Sequence Typing) method and a complete sequencing of the genome of each strain will be performed to provide a detailed view of the evolution of the strains in Morocco and Algeria. These studies will be supplemented by a phenotypic analysis on the expression of antigens in order to predict the coverage of these strains by new vaccines.

This project is expected to provide important results for understanding the epidemiology of invasive meningococcal infections, vaccination coverage of different vaccines and also building the basis for future vaccination recommendations.

### Non-invasive Diagnostic Test for Early Identification of Arbovirus Strains (Arbovirtuess)



#### Project ACIP n° 04-2014

**Coordinator:** Nancy ROOSENS–Platform Biotechnology and Molecular Biology – Scientific Institute of Public Health (Brussels, Belgium)

**Institut Pasteur International Network collaborators:** Dr Steven VAN GUCHT, Scientific Institute of Public Health (Brussels, Belgium), Dr Laure DIANCOURT, Institut Pasteur (Paris), Dr Ann-Claire GOURINAT and Dr Myrielle DUPONT-ROUZEYROL, Institut Pasteur in New Caledonia and Dr Dominique ROUSSET, Institut Pasteur in French Guiana

#### **THE CONTEXT**

Vector-borne diseases account for more than 17% of all infectious diseases, causing more than 1 million deaths annually.

Invasive samples as blood or cerebrospinal fluid are required for currently used diagnostic tests for arboviruses.

#### **THE PROJECT**

The project aims at developing an acid-nucleic based test to specifically detect arboviruses on saliva and urine. The test will be adapted to Multiplex technology (i.e.: xMAP, Luminex®). The test targets the Dengue virus (serotypes 1-4), the Yellow fever virus, the tick-borne and Japanese encephalitis viruses, the Zika virus, the Chikungunya virus, the Ross river virus and the West Nile virus.

Expected advantages are following: several arboviruses can be tested in a single run, at the early phase of the infection and even without specific symptoms. This tool is easily adaptable to new targets such as (re)emergent pathogens.

The sensitivity and the specificity of the test will be obtained from cohorts of 350 positive and negative patients per arbovirus of interest at the Institut Pasteur of French Guiana and New Caledonia.

As an additional or alternative laboratory diagnostic test to immunological assays and qRT-PCR techniques already used, this technology will improve the early diagnosis and survey of arboviruses strains, thus allowing a faster orientation of the patients during an outbreak. Acid-nucleic based tests are highly specific compare to serological-based ones.

Non-invasive diagnostic test for early detection & viral identification of arbovirus infection.

#### **PUBLICATIONS:**

- Barbau-Piednoir E., Roosens N.H. et al., SYBR®Green qPCR Salmonella detection system allowing discrimination at the genus, species and subspecies levels. Applied Microbiology and Biotechnology, 2013
- Desdouits M, Manuguerra JC et al., Genetic characterization of Chikungunya virus in the Central African Republic, Infection, Genetics and Evolution, 2015
- Berthet N, Manuguerra JC et al., Molecular characterization of three Zika flaviviruses obtained from sylvatic mosquitoes in the Central African Republic, Vector-Borne and Zoonotic Diseases, 2014

### Development of standardized clinical and genetic diagnoses for autism spectrum disorders to better investigate the genetic causes of these disorders



#### Project ACIP n° 07-2014

Scientific coordinator: Pr Thomas BOURGERON, Human Genetics and Cognitive Functions Unit - Institut Pasteur in Paris Institut Pasteur International Network collaborators: Dr Abdelhamid BARAKAT, Institut Pasteur in Morocco, Dr Sonia ABDELHAK, Institut Pasteur in Tunis \*

#### WHAT ARE AUTISM SPECTRUM DISORDERS?

Autism Spectrum Disorders (ASD) are neurological disorders that affect many aspects of child development. They are characterized by impairments in social interactions and by the presence of stereotyped and restrictive patterns of interest and behavior.

The level of intellectual functioning is extremely variable and can range from profound impairment to higher cognitive abilities. Even in individuals without intellectual disability, difficulties in social interaction and in adaptative behavior can lead to difficult schooling, an inability to live independently from parents, and a lack of employment as adults. ASD thus has consequences at an individual and at a societal level. No treatment has proven efficient so far, but clinical and genetic strategies must be implemented during early childhood to permit an early detection of ASD that can help setting up an environment that will favor the development and well-being of the individual with ASD.

#### WHAT ARE THE GENETIC CAUSES OF AUTISM SPECTRUM DISORDERS?

Twin and family studies have conclusively described ASD as the most « genetic » neuropsychiatric disorder. It is now established that autism symptoms can be caused either by gene mutations or by chromosomal aberrations. In the last years, several independent studies and large-scale international efforts have identified genetic variations associated with ASD such as rare variants, copy number variants (CNVs) and single nucleotide polymorphisms (SNPs).

The discovery of treatments is hampered by the heterogeneity of ASD. All mutations identified so far account for a small subset of patients (<1%). As a consequence, it is difficult to ascertain a robust genotype-phenotype relationship. One way to better understand the genetic susceptibility to ASD is therefore to analyze gene pathways instead of single genes and to study large samples of patients in order to reach sufficient statistical power to identify subgroups of patients with ASD. Such large cohorts are however often heterogenous in their clinical and genetic diagnoses are not always standardized. It is therefore crucial to implement these standardized diagnoses in order to carry out studies on large homogeneous cohorts to better investigate the genetic causes associated with ASD.

ASD affect 1% to 2% of the population (3 millions individuals in the European Union alone) and represent an important problem for many families, as well as for public health and educational systems.

The prevalence of ASD is about 1/100 overall, but closer to 1/300 for typical autism.

#### **THE PROJECT**

This collaborative project gathering psychiatrists and geneticists from the Institut Pasteur in Paris, Tunisia and Morocco aims to provide a better diagnosis and care of patients with ASD. It will allow the mutual sharing of experiences between the various teams involved. First, the psychiatrists, from Paris, Morocco and Tunisia will create and implement a common standardized clinical assessment battery at each participating center, recruiting patients with ASD and their relatives in Morocco and Tunisia. Then, the geneticists will explore a group of patients from the 3 countries to set up a standardized genetic diagnosis for ASD and identify new genes associated with ASD (with a focus on recessive genes in consanguineous families). The standardized genetic diagnosis will include the identification of CNVs and mutations in the main genes associated with ASD thanks to new genetic approaches such as high-throughput genotyping and next generation sequencing.

During this project, standardized clinical and genetic diagnosis will be developed in order to provide homogeneous and well-phenotyped cohorts of ASD patients and their relatives. This project, conceived as a pilot study, could be extended to other countries in the Mediterranean region.

The study of well phenotyped cohorts of patients with ASD will allow to deepen the genetic knowledge of ASD and identify treatments. Annual care costs for a child with ASD have been estimated 85-550% higher than for a typically developing child.

### A better understanding of dengue circulation mode in cities in Southeast Asia through surveillance tools to develop transmission models and prevent new emergence



#### Project ACIP n° 09-2014

Scientific coordinator: Dr Marc GRANDADAM and Dr Sompavanh SOMLOR, Arbovirus and Emerging viral diseases laboratory - Institut Pasteur in Laos Institut Pasteur International Network collaborators : Dr Valérie CARO, Institut Pasteur (Paris), Dr Philippe BUCHY, Institut Pasteur in Cambodia \*

#### **DENGUE IN SOUTHEAST ASIAN COUNTRIES**

Dengue is a viral vector disease that is transmitted by mosquito bites. Mosquitoes of the *Aedes* genus, and more particularly *A. aegypti* and *A. albopictus*, are the two main mosquito vectors of the dengue virus.

Southeast Asia is considered a hyperendemic area for dengue fever. The impact of this disease is explained by various factors as diverse as the circulation of the four serotypes of the virus in a hyperendemic mode, the presence of two main species of mosquitoes vector dengue viruses, the lack of means of early diagnosis and taking in fast charge of severe forms.

To date, there is no treatment or vaccine, the only prevention of epidemics is based on the application of individual and collective vector control measures. The destruction of breeding sites or the application of larvicides and adulticides are organized during the season of maximum activity of the vectors.

#### **DENGUE SURVEILLANCE NETWORKS**

Before 2012 in Laos, dengue surveillance was based solely on reports of suspected cases based on the WHO clinical classification. On the initiative of the Pasteur Institute of Laos, coordination of dengue surveillance was set up. This program has strengthened the capacity for virological diagnosis of dengue fever in Laos, centralized the data of the various institutions and set up a weekly surveillance report transmitted to the Ministry of Health of Laos. This longitudinal monitoring showed the seasonal nature of dengue fever reaching its peak between August and September but also an effective circulation in the dry season associated with the occurrence of deadly forms of dengue fever. Studies have been undertaken several countries in Asia to explain the persistence of dengue viruses during the inter-epidemic period. These first works focus in general on a parameter (vector dynamics, genetic polymorphism of viruses,...) and then try to make a retrospective link with the epidemiology of dengue fever in the studied region. The mechanisms leading the endemic condition of dengue in Laos are not known. It may be the result of a single cause but it seems more likely that it results from the combination of several factors. Identifying these factors at a local scale is essential to consider effective control of dengue transmission. The local specificities make it difficult to generalize the measures of prevention of the dengue by the vector control, as well from the point of view of the period as of the means to be implemented.

Dengue is the vector-borne viral disease that has the greatest impact on human health because of its mortality and morbidity.

According to WHO, more than 100 countries, with at least 2.5 billion people, are affected by this virus.

#### **THE PROJECT**

The scientists from Institut Pasteur in Laos, Cambodia and Paris will combine their complementary expertise to understand the circulation modes of dengue using surveillance tools previously developed by Institut Pasteur in Laos and Cambodia in Southeast Asian cities in order to predict new epidemics. This project can be achieved through the collaboration with surveillance networks, involving health care institutions, present in Laos and Cambodia. The scientists will identify the mosquito species involved in the dengue virus transmission during the dry season. They will study the genetic polymorphism of dengue strains in order to understand the mechanisms of escape of the viruses. Then they will establish a commun collection of the dengue strains to refine the knowledge on trafficking of dengue strains in Laos and in the sub-region. The team will model the flow of dengue in the capitals of Laos and Cambodia using data already acquired by the dengue surveillance networks.

The project will provide new knowledge on the dengue circulation in Southeast Asia countries. It will also strengthen the links between the institutes, involved in this project, creating a dengue based sentinel network that can be expanded to other arboviruses to detect emergences and to make local recommendations. The countries of Asia, and in particular those of the Indochinese peninsula, suffer serious damage for this infection.

### Diagnostic, molecular evolution and vector competence of Zika virus in Africa, Asia and the Pacific



#### Project ACIP n° 15-2014

**Coordinators:** Dr Myrielle DUPONT-ROUZEYROL, URE Dengue & Arboviruses - Institut Pasteur in New Caledonia and Dr Oumar FAYE, Arbovirus and Hemorrhagic Viral Fever Unit - Institut Pasteur Dakar **Institut Pasteur International Network collaboration:** Dr Laurent GUILLAUMOT, Institut Pasteur in New Caledonia, Dr Philippe BUCHY, Institut Pasteur in Cambodia, Dr Marc GRANDADAM, Institut Pasteur in Laos, Dr Anna-Bella FAILLOUX, Institut Pasteur (Paris) \*

#### **THE CONTEXT**

Molecular diagnostic tools currently used do not take into account various geographical origins of Zika virus strains. Few have been evaluated on human samples.

#### **THE PROJECT**

This project proposes to standardize diagnostic methods currently used for Zika virus detection, by assessing currently available tests with sera from clinically infected patients and taking into account the genetic diversity of the virus worldwide. In parallel, vector competence studies based on several Aedes mosquitos' populations will be undertaken.

Expected advantages are following: improved sensitivity, repeatability and reproducibility of the diagnostic tools, study of virus macro-evolution in all world regions where the virus is present.

The scientists will be used a panel of serum and mosquitoes naturally infected from Africa, Asia and the Pacific to standardize and improve the currently diagnostic methods.

This project aims at improving diagnostic tools of the Zika virus for a better monitoring and management of patients. Moreover, studies on the viruses and the vectors will allow a better understanding of (i) the emergence mechanisms underlying an outbreak episode, (ii) the origin of outbreaks and (iii) the ability of the virus to be transmitted by specific mosquito's species and to persist into the wild.

\* This project also involves / involved the participation of external partner(s).

In November 2015, the Brazilian Ministry of Health declared a public health emergency concerning the possible relationship between the unusual increase in the number of children born with microcephaly and the ongoing Zika outbreak.

Standardization of Zika diagnostic methods taking into account various geographical origins of Zika virus strains.

#### **PUBLICATIONS:**

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# Hepatitis E in Cambodia and Vietnam: Is it emerging?



#### Project ACIP N° 03-2015

**Coordinator:** Dr Francois ROUET, HIV / Hepatitis Unit - Institut Pasteur in Cambodia **Institut Pasteur International Network collaborators:** Dr Ton TRAN, Institut Pasteur in Ho Chi Minh City (Vietnam), Dr Yoann MADEC, Institut Pasteur (Paris) \*

#### WHAT IS HEPATITIS E?

The Hepatitis E is a liver disease caused by the Hepatitis E virus (HEV), a causative agent considered as an important public health concern in many parts of the world with 20 million cases recorded each year. The Hepatitis E is transmitted in developing countries mainly by contaminated drinking water and generally gives rise to an infection that spontaneously regresses in 4 to 6 weeks. Occasionally, a fulminant form of Hepatitis E (acute liver failure) may occur and results in death. HEV have also become an emerging problem in developed areas, with a number of sporadic cases linked to exposure to pigs and consumption of undercooked pork, wild game and offal (foodborne transmission). Case reports have also linked Hepatitis E to consumption of shellfish.

#### AVAILABLE DATA ABOUT THE EPIDEMIOLOGY OF THE HEPATITIS E

Southeast Asia is often considered as a highly endemic region for Hepatitis E occurring in humans. However, available data are scant, sometimes old, and display significant regional variations according to the 11 countries of the region. In Cambodia, there are only two studies on HEV seroprevalence in humans, and human HEV strains circulating in Cambodia are unknown. Today there is no treatment to influence the trend of acute Hepatitis. Prevention is therefore the most effective approach against the disease, and preventive strategies need strong data in epidemiology.

#### **THE PROJECT**

This study, conducted by Dr. François ROUET, will provide an update on the past and current magnitude of HEV seroprevalence in Cambodia and Vietnam. It will extend the knowledge on prevalence rates of Hepatitis E among urban population groups from Cambodia and Vietnam. The teams will investigate if HEV prevalence rates are increasing, decreasing or stable over time in these two settings. Moreover, this study will allow assessing the risk for foodborne infections: for this purpose, pig products and shellfish from Cambodia and Vietnam will be tested for HEV.

More than 3 million of acute Hepatitis E cases are reported each year worldwide.

The Hepatitis E is spread through fecal-oral route, and mainly through contaminated water.

The prevalence of Hepatitis E is highest in East and South Asia.

# Biomarkers to identify patients with high risk of Gastric cancer



#### Project ACIP n° 10-2015

**Coordinator:** Dr Eliette TOUATI – Unit of Helicobacter Pathogenesis – Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Marjan MOHAMMADI, Institut Pasteur in Iran and Dr Fatima MAACHI, Institut Pasteur in Morocco \*

#### **THE CONTEXT**

Gastric cancer is of multifactorial origin, but epidemiological investigations have clearly established that *Helicobacter pylori* (Hp) infection is the major risk factor for gastric cancer. This bacterium is indeed responsible for the most common chronic infection with approximately half of the world's population infected, with a higher prevalence of 70-80% in developing countries. Often detected at an advanced stage, gastric cancer affects about one million people per year and is associated with 800,000 deaths with a higher incidence in males. Wide geographic variation in survival has been reported, depending mainly on differences in stages of diagnosis. Indeed, if it is diagnosed at an early stage, the disease can be cured. However, no appropriate screening strategies are available for a global application to reduce the global burden of gastric cancer.

#### **THE PROJECT**

This project will deliver specific biomarkers of gastric cancer lesions in order to identify patients with high risks to develop this disease.

The biomarkers candidates will be identify after a study performed on three cohorts of patients at each stage of the gastric carcinogenesis cascade and from different geographical origins: West Asia, North Africa and West Europa, countries with higher incidence of cancers.

Expected advantages of these biomarkers are following: (i) to detect the gastric cancer in absence of clinical symptoms and to deliver the most precise screening of the populations affected, (ii) to allow the development of non-invasive test, (iii) to monitor disease remission and progression during an anti-cancer treatment.

The identification of biomarkers candidates for gastric cancer will have a strong impact in clinic and will pave the way for the development of a non-invasive test for early detection and diagnosis of patients with high risk of gastric cancer.

\* This project also involves / involved the participation of external partner(s).

Gastric cancer is the fourth most common malignancy and the third cause of cancer relateddeath worldwide.

Characterization and validation of biomarkers to detect earlier the presence of gastric cancer lesions.

#### **PUBLICATIONS:**

- Matak P, Touati E et al., Myeloid HIF-1 is protective in Helicobacter pylori-mediated gastritis, Journal of immunology, 2015
- Fernandes J, Touati E et al., Circulating mitochondrial DNA level, a noninvasive biomarker for the early detection of gastric cancer, Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 2014
- Correia M, Touati E et al., Crosstalk between Helicobacter pylori and gastric epithelial cells is impaired by docosahexaenoic acid, Plos One, 2013

# Identify regulators associated with major alterations observed in diseased muscle



#### Project ACIP nº 13-2015

**Coordinator:** Dr Shahragim TAJBAKHSH, Stem Cells and Development Unit – Institut Pasteur (Paris) **Institut Pasteur International Network collaborators:** Dr Houda YACOUB and Sonia ABDELHAK, Institut Pasteur in Tunis, Dr Loubna MAZINI, Institut Pasteur in Morocco \*

#### WHAT ARE MUSCULAR DYSTROPHIES?

The muscular dystrophies (MD) are a group of more than 30 genetic diseases characterized by progressive weakness and degeneration of the skeletal muscles that control movement. Naturally, skeletal muscle regeneration is effected by muscle stem (satellite) cells, which are located beneath the basal lamina of the myofibre. In resting muscles satellite cells are quiescent, and following injury, they activate, proliferate, migrate and differentiate to fuse with each other or to existing myofibres.

#### DETERMINE THE MOLECULAR PATHWAYS INVOLVED IN MUSCLE HOMEOSTASIS

Considerable progress has been made in understanding gene regulatory networks that regulate myogenic stem cell emergence. Recent studies have underscored the important role of specific regulators involved in different stages during skeletal myogenesis in different organisms. In spite these progress in model organisms such as the mouse, it is not clear to what extent these important regulators act during muscle homeostasis and regeneration or disease, in particular in the context of the healthy and pathological muscle microenvironnement, specifically in humans.

#### **THE PROJECT**

The proposed collaboration is the first "pilot study" on the investigation of muscle diseases in the Institut Pasteur International Network. This study will involve teams from three Institut Pasteur that will work together to advance their understanding of stem cells in the context of muscle disease. They will assess at a molecular and cellular level the key regulators involved in myogenesis in healthy muscles, compared to diseased human muscles. The long-term objective is to provide molecular alterations (biomarkers) present in the pathogenesis of Duchenne Muscular Distrophy and in Limb Girdle Muscular Dystrophy, in collaboration with the three Institutes.

Duchenne Muscular Dystrophy is the most common form of Muscular Dystrophy.

In the US in 2007, about 15 out of every 100,000 males ages 5-24 years were affected by Duchenne or Becker muscular dystrophy (DBMD).

Today, there is no cure for any form of muscular dystrophy.

# Drug discovery to treat the neglected area of trypanosomatids



#### Project ACIP nº 17-2015

**Coordinator:** Dr Marcelo COMINI, Laboratory Redox Biology of Trypanosomes – Institut Pasteur in Montevideo Institut Pasteur International Network collaborators: Dr Joo Hwan NO, Institut Pasteur Korea, Dr Camila INDIANI DE OLIVEIRA, FIOCRUZ (Brazil) \*

#### WHAT ARE TRYPANOSOMATIDS?

Trypanosomatids are pathogenic parasites responsible for three major human diseases: African trypanosomiasis (sleeping sickness, caused by *Trypanosoma brucei* and transmitted by tsetse flies), South American trypanosomiasis (Chagas disease, caused by *Trypanosoma cruzi* and transmitted by triatomine bugs), and leishmaniasis (a set of trypanosomal diseases caused by various species of *Leishmania* transmitted by sandflies). Extended in the tropical and subtropical regions of the different continents, these diseases altogether affect about 30 million of people and account for half a million of fatalities per year. They also cause subsantitial economic problems in endemic areas by affecting livestock.

#### A VERY LOW THERAPEUTIC ARSENAL

The available therapeutic arsenal for infectious diseases caused by protozoan parasites of the trypanosomatid family is unsatisfactory in terms of safety and efficacy. Indeed, they suffer from limited number of drugs available that displaying low efficacy, high toxicity and non-easy administration, and clinical phase is rather limited. Otherwise, there is no prospect for the generation of vaccines due to complex mechanisms used by the parasites to evade the host immune response. Chemotherapy therefore remains as the most reliable choice for combating these pathogens but the portfolio of candidates in pre-clinical and clinical phases is rather limited. There is therefore an urgent need to discover new entities with potent and selective anti-trypanosomatids activity and clinical standards of safety.

#### **THE PROJECT**

The present project aims at paving the way for the identification and characterization of novel target-specific drug-like compounds to treat neglected diseases caused by trypanosomatids (African sleeping sickness, Chagas disease and Leishmaniasis). Dr. Marcelo Comini, at the head of the project, as well as the other members cover all expertise required for the multidisciplinary early drug discovery phase and have already contributed to the identification and characterization of compounds with anti-parasitic activity. The objective is to deliver novel drug candidates against trypanosomiasis and leishmaniasis with a known mode of action and suitable biological activity to initiate clinical studies and/or preclinical tests.

Chagas' disease and Animal Sleeping sickness cause 500,000 to 1 million deaths annually.

Chagas' disease, caused by *Trypanosoma cruzi*, is the third largest disease burden in Latin America.

Leishmaniasis threaten about 350 million people in 98 countries or territories in the world.

### Identification of broad-spectrum naturally derived inhibitors against hepatotropic viruses (Dengue, Yellow fever and hepatitis B)



#### Project ACIP n° 18-2015

**Coordinator:** Dr Niki VASSILAKI, Molecular Virology Laboratory - Hellenic Institut Pasteur (Athens, Greece) Institut Pasteur International Network collaborators: Dr Christine NEUVEUT, Institut Pasteur (Paris), Dr Marc P. WINDISCH, Institut Pasteur Korea \*

#### **DENGUE, YELLOW FEVER AND HEPATITIS B: HEPATOTROPIC VIRUSES**

The mosquito-borne Dengue virus (DENV) and Yellow fever virus (YFV) are reemerging global pathogens causing life-threatening haemorrhagic fevers. The hepatitis B virus (HBV), another hepatotropic agent, is the world's leading cause of serious liver complications like cirrhosis and hepatocellular carcinoma. Today, there is no approved specific therapy to treat DENV and YFV. Concerning HBV, current standard of care is not curative for the vast majority of the patients and consequently requires lifelong and high cost therapy. Moreover today, all available treatments target the same mechanism to inhibit the HBV. There is therefore a significant unmet medical need for new safe effective and affordable DENV, YFV and HBV drugs.

#### THE URGENT NEED FOR IDENTIFYING NEW DIRECT-ACTING ANTIVIRALS

Today, in spite of the global threat of viral pandemics caused by the reemerging agents DENV and YFV, there is no clinically approved specific therapy to treat DENV and YFV. Many of the inhibitors of DENV and YFV have not advanced beyond the stage of hit-to-lead optimization to clinical development, due to their poor selectivity, physiochemical or pharmacokinetic properties and selection of drug resistance-associated mutations. Development of prophylactic and/or therapeutic treatment against DENV, YFV and HBV infections using natural products may help address some of these current limitations.

#### **THE PROJECT**

The Instituts Pasteur of Greece, Paris and Korea and external partners will take advantage of their synergistic expertise on the discovery of new safe, broadly effective and affordable antiviral candidate drugs in natural products and derivatives against Dengue, Yellow fever and hepatitis B viruses. They will employ a cell culture model for virus replication, based on the median oxygen tension of hepatic microenvironment and stimulating metabolic conditions of liver disease. For this antiviral screening the Instituts Pasteur will evaluate against Dengue, Yellow fever and hepatitis B viruses two small compound collections: 1) A series of fungal metabolite analogues which are structurally related to chemicals previously shown as broadly effective against the replication of hepatitis C, HIV and Influenza viruses. 2) A group of natural products isolated from plants of the Greek and Mediterranean biodiversity that have been previously recognized as suppressors of hepatitis C replication. Then, modes of action of the most promising candidates will be elucidated.

Hemorrhagic fevers caused by DENV and YFV can be lifethreatening when left untreated.

Although vaccination programs, chronic hepatitis B still affects 350-400 million people worldwide.

### Characterization of the insecticide susceptibility of natural populations of *Aedes Aegypti* and *Aedes Albopictus* mosquitoes to determine mechanisms involved in resistance to insecticides



#### Project ACIP n°22-2015

**Coordinator:** Dr Carine NGOAGOUNI, Virology and Medical Entomology Service - Institut Pasteur in Bangui Institut Pasteur International Network collaborators: Dr Sébastien BOYER, Institut Pasteur de Madagascar, Dr Isabelle DUSFOUR, Institut Pasteur in French Guiana \*

#### WHO ARE AEDES AEGYPTI AND AEDES ALBOPICTUS?

Aedes aegypti and Aedes albopictus are the main epidemic vectors of dengue and chikungunya worldwide. Dengue virus and chikungunya virus are mosquito-borne viruses of medical concern in most tropical and subtropical regions. Both mosquito-borne-virus, dengue and chikungunya, are becoming an increasingly important global health threats, spreading from their original niche in sub-Saharan Africa to most areas of the world. Dengue fever is characterized by clinical manifestations range from mild cases to severe cases of dengue hemorrhagic fever (DHF) and/ or dengue shock syndrome (DSS). Chikungunya fever is characterized by distinct forms, asymptomatic, classical and severe (such as neurological or cardiovascular disorders).

#### THE FIGHT AGAINST THE MOSQUITO VECTORS OF DENGUE AND CHIKUNGUNYA

In the absence of an effective vaccine and specific treatment against dengue and chikungunya, vector control remains the primary method of intervention and outbreak control. Unfortunately, long term and intensive use of insecticides in agriculture and public health in several regions through the world often lead to emergence of resistance, which is one of the major obstacles in the control of medical and agricultural arthropod pests. Many mosquito vector control programs are threatened by the development of insecticide resistance in these both species. Moreover, there is an absence of data on the evaluation of the sensitivity to different molecules (case of Madagascar and Central African Republic) used in public health against these two vectors and/or gradual withdrawal of some of them (case of French Guiana) represent a growing obstacle for dengue and chikungunya control programs in these three territories.

#### **THE PROJECT**

This study will investigate the susceptibility of the two main vectors of dengue and chikungunya to insecticides commonly used, in order to implement effective and sustainable arbovirus vector control measures. Dr. Carine Ngoagouni and her team will also explore the mechanisms involved in adult stages of *Aedes aegepti* and *Aedes albopictus* insecticide-resistance to guide knowledge of the best choice of chemical for use in the event of dengue and chikungunya outbreaks. Dengue virus infection is a serious health problem. 2.5 billion people wordlwide are exposed to dengue virus.

In 2005, a massive outbreak of chikungunya in the island of a Reunion affected nearly 40% of the population.

No specific treatment exists to defeat dengue or chikungunya.

### Better knowing the environment of the Aedes aegypti mosquito's breeding places, in order to better control it



#### Project ACIP n° 01-2016

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#### **ARBOVIRUSES IN THE FRENCH TERRITORIES OF THE AMERICAS**

Dengue, Zika and Chikungunya viruses are arboviruses that are transmitted to humans by female mosquites of the genus *Aedes*. These arthropods act as a vector which usually constitutes the viral reservoir. Infection occurs via the bloodstream, alternately from vector to vertebrate and vertebrate to vector. Humans are not the only vertebrate hosts—there are other mammals (monkeys, rodents, bats, domestic animals), birds, amphibians and reptiles. Direct person-to-person transmission does not usually occur, except via blood transfusions, and in certain cases from mother to infant.

Arbovirus infections cause a variety of symptoms due to the fact that they belong to different families of viruses.

For the last 30 years, the French territories in the Americas have been facing dengue epidemics of increasing frequency and number of cases with fatal outcomes. Moreover, these territories were affected by chikungunya virus in 2013-2014, and Zika virus in 2015-2016, with the latter causing large-scale epidemics in Guadeloupe, Martinique and French Guyana.

#### **MOSQUITO BREEDING PLACES**

The species of mosquito, *Aedes aegypti*, that carries these viruses is highly abundant in these territories, and traditionally reproduces in containers of clean water. Recently, these mosquitoes have also been seen to colonise containers of water rich in suspended particles (dirty water). The bacterial communities and physicochemical properties of these two types of breeding site are perhaps different (due to variations in UV, and exposure to nutrients and oxygen). All these elements can influence the intestinal microbiota of *Aedes aegypti*, and indirectly, some of the mosquito's characteristics, such as insecticide resistance, survival and vector competence to transmit dengue, chikungunya and Zika viruses.

A hypothesis has been proposed according to which *A. aegypti* mosquitoes that develop in "clean" breeding sites are more "dangerous" than those that develop in "dirty" water with respect to arboviral transmission.

#### **THE PROJECT**

This consortium, made up of teams from Guadeloupe, French Guiana and Canada, proposes to study the role of the microbiota in survival of mosquitoes and their competence to transmit dengue and Zika viruses. This is one of the first studies to allow investigation of the effect of the vector's microbiota on its competence to transmit Zika virus.

To do this, they will identify the bacterial communities in water from the "clean" and "dirty" breeding sites used by the mosquitoes, and determine the physicochemical characteristics of these waters. This study will make it possible to identify the bacteria from the mosquito's microbiota that have a major influence on the mosquito's survival or competence to transmit dengue and Zika viruses.

This knowledge will make it possible to establish new strategies for the biological control of *Aedes* populations, and hence limit the spread of the diseases transmitted by this mosquito.

This project is a pilot study which, if it turns out to be conclusive, may be extended to chikungunya virus and other institutes in the network.

\* This project also involves / involved the participation of external partner(s).

Mosquitoes that spread Zika, chikungunya virus bite during the day and night.

In April 2015, more than 1,379,788 chikungunya suspected cases had been recorded in the Caribbean and American continent.
# Better understanding dengue virus evolution to better protect populations



### Project ACIP n° 06-2016

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### DENGUE

Dengue is a viral infection transmitted by female mosquitoes of the genus *Aedes*. The two vectors responsible are *Aedes aegypti*, the main vector, and *Aedes albopictus*, the Asian tiger mosquito.

It is endemic in tropical and subtropical regions throughout the world, with a predilection for urban and semi-urban areas. For some years, cases of dengue have been observed in temperate areas due to colonisation by the Asian tiger mosquito.

This infection causes an influenza-like syndrome, and affects infants, young children and adults. In some patients, the disease can progress to serious forms, namely haemorrhagic dengue and dengue shock syndrome, which can lead to death, especially in children.

Currently, 4 serotypes of dengue virus are recognised. Recovery confers lifelong protection from the serotype that caused the infection. There is no cross-immunity between serotypes. Subsequent infection by other serotypes increases the risk of developing a severe form of the disease.

A vaccine for dengue has been authorised in Mexico, the Philippines and Brazil for the prevention of this disease in the 9- to 45-year old population. It confers a 65% rate of protection, and will be added to the prophylactic measures.

### **EVOLUTION OF THE DENGUE VIRUS**

Dengue epidemics have a particular dynamic. Genetic studies of dengue viruses involved in these epidemics have shown that the dynamic of these epidemics is frequently related to the replacement of a dengue genotype by another genotype of the same serotype.

There is a need to understand the causes of these changes, which are important both epidemiologically and in combating dengue, since they are often associated with changes in disease severity and in the ability of the human body to defend itself against the virus. These mechanisms for genotype replacement during an epidemic have received little attention.

### **THE PROJECT**

The Institut Pasteur teams from New Caledonia, Cambodia and Paris propose to combine their complementary expertise to achieve a better understanding of the evolutionary mechanisms that lead to the genotype replacement generally observed in dengue virus during epidemics of this disease. To do this, the researchers will characterise dengue viruses from New Caledonia and Cambodia, evaluate the potential role of selection caused by the vector, and also measure the replicative abilities of genotypes of the dengue virus *in vitro*.

The results will help to provide a better understanding of the evolutionary mechanisms leading to replacement of dengue viruses during epidemics. This new knowledge will be highly useful in vaccine design. Indeed, the strains of dengue virus resulting from this evolution may have different antigenic properties. These elements need to be taken into account when creating a vaccine, in order to obtain maximum protection for the population.

\* This project also involves / involved the participation of external partner(s).

The severe form is unpredictable and represents about 1% of cases, most often in children under 15.

World Health Organization estimates 50 million cases annually, including 500,000 cases of haemorrhagic dengue.

# Better understanding the viruses that cause hand, foot and mouth disease in order to improve its control



### Project ACIP n° 22-2016

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### WHAT IS HAND, FOOT AND MOUTH DISEASE?

Hand, foot and mouth disease is a highly contagious viral disease that affects children under 10 years, but also affects adults. The disease occurs all over the world. It is caused by enteroviruses, usually Coxsackie virus A-16 or enterovirus 71 (EV-71).

It is transmitted by direct contact between infected children, and is characterised by fever, and the development of spots in or on the mouth, hands, feet, and buttocks. The disease is usually mild, with spontaneous recovery in 7-10 days. However, severe complications can occur, with neurological, cardiac and respiratory involvement, and may be fatal.

Transmission of enteroviruses is favoured by factors such as poor hygiene conditions and overcrowded housing.

To date, there is no specific treatment or vaccine available.

### **ENTEROVIRUS 71 IN AFRICA**

Serious or even fatal complications (neurological, cardiac and respiratory) are caused by enterovirus 71.

Since the 1980s, the majority of epidemics caused by EV-71 have taken place in Asia. In the last 15 years, EV-71 has continued to circulate in Africa, Europe and the American continent, sporadically causing small-scale epidemics.

EV-71 has been detected in certain clinical samples in Cameroon, the Central African Republic, Madagascar, Senegal, Mauritania, Guinea-Conakry and Niger. However, there is no published data available on the incidence of hand, foot and mouth disease in Africa, where this infection is not a notifiable infection, and does not have a public health surveillance program.

### **THE PROJECT**

The Institut Pasteur teams from Dakar, Bangui and Paris will combine their expertise to study the causes and cases of hand, foot and mouth disease present in paediatric structures in Senegal and the Central African Republic. Another objective of this project will be to understand and describe the epidemiological and genetic characteristics of the enteroviruses associated with this disease.

This project will make it possible to obtain epidemiological information on this disease, along with data on the viruses responsible for this disease in the countries of Africa.

This new knowledge could help to determine precautionary measures for better management of future epidemics, and to define a multivalent vaccine combining several enteroviruses.

Strengthening hygiene is important in preventing.

Neurological consequences and even death are possible in the infant.

# Better understand the physiopathological mechanism of diabetes and identify biomarkers to improve diagnosis and prevention

# ACIP SINSTITUT

### Project ACIP n° 45-2017

**Scientific coordinator:** Dr Rym KEFI, Laboratory of Biomedical Genomics and Oncogenetics - Institut Pasteur in Tunis **Institut Pasteur International Network collaborators :** Dr Charles RAMASSAMY, INRS – Institut Armand Frappier (Canada), Dr Philippe SANSONETTI, Institut Pasteur (Paris) \*

### WHAT IS TYPE 2 DIABETE?

Type 2 diabete (T2D) is characterized by a chronic hyperglycemia, that is an excess of sugar in the blood and thus a glucose level (glycemia) too high. This chronic disease occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar.

T2D is a global public health problem because the number of affected people is growing steadily. Until recently, T2D was seen only in adults but it is now also occuring increasingly frequently in young individuals.

T2D is rarely accompanied by symptoms in the beginning. As a result, the disease may be diagnosed several years after onset, once complications have already arisen. Indeed, in the long term, chronic hyperglycemia causes degenerative organic complications particularly affecting the eyes, kidneys, nerves, heart and vessels. Treatment of T2D involves initially diet and physical activity before using antidiabetic treatments.

T2D is strongly linked to obesity, physical inactivity but also to several diseases as metabolic syndrome, cancer, cardiovascular diseases, cognitive declin and dementia.

T2D constitutes a socio-economic burden in terms of quality of life, mortality and health cost.

### WHAT ARE THE FACTORS INVOLVED IN TYPE 2 DIABETE?

T2D is a complex disease due to many causes involving genetic and environmental factors such as lifestyle (dietary practices and physical activity). It has been shown recently that other elements such as microbiota and oxidative stress may have a role in the pathophysiology of T2D. An unbalanced flora affects homeostasis and maintenance of the integrity of the intestinal barrier which causes several diseases such as T2D, obesity and cancers. Oxidative stress corresponds to the disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses which is at the origin of ageing and may play a crucial role in T2D. Some studies suggested mechanisms linking hyperglycemia and oxidative stress.

This multifactorial aspect represents the major source of difficulty in understanding the causal mechanisms of T2D. The involvement of these various factors in T2D has been established, on the other hand their interaction and their role have not yet been fully elucidated in the occurrence of degenerative organic complications and cancer in T2D patients.

Diabetes represents a growing public health problem affecting currently 415 million people worldwide.

Diabetes prevalence has been rising more rapidly in middleand low-income countries.

The consortium of this project (DiaBiomark project) composed by three teams from IPIN (Tunis, Paris and Canada) will combine their complementary expertise to set up a multidisciplinary study to better understand the pathogenesis of T2D. They propose to decipher the interactions between the various factors involved in the development of T2D and its associated pathologies in Tunisian population in order to identify biomarkers with diagnostic value. For this, they will collect biological samples and epidemiological, clinical, and genealogical data from differents categories of T2D patients as well as healthy controls in Tunisia. From the biological samples, they will determine, genetic, stress oxidative and microbiotic profiles of these patients and controls. And then, they will combine and analyze all these data in order to identify differents biomarkers linked to T2D.

These data will allow to better understand the physiopathological mechanism of diabetes and to identify new biomarkers. These will improve the diagnosis of T2D, better monitor its evolution and prevent its complications. This will improve the quality of life of the patient and reduce the cost of his care and will thus have significant socio-economic benefits.

In 2015, an estimated 1.6 million deaths were directly caused by diabetes.

# Better understand the impact of the gut microbiota on the liver diseases in order to prevent and slow them down



Scientific coordinator: Dr Pascal PINEAU, Nuclear Organization and Oncogenesis Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Soumaya BENJELLOUN, Institut Pasteur in Morocco, and Dr Farzin ROOHVAND, Institut Pasteur in Iran \*

### LINK BETWEEN METABOLIC DISORDERS AND LIVER DISEASES IN MIDDLE EAST AND NORTH AFRICA

The Middle East and North Africa (MENA) is known as one of the regions with the highest rates of obesity and type 2-diabetes. An increase of these **metabolic diseases** is due to the changes affecting the way of living including urbanization and nutritional transition. They are currently affecting MENA populations as early as infancy or childhood, a situation predicted to generate deleterious health consequences for the future grown-up generations.

Metabolic disorders emerged recently as a major cause of morbidity for the liver and they represent important risk factors for terminal diseases. **Terminal liver diseases** include Cirrhosis and Hepatocellular carcinoma that are respectively an inflammation of the liver with gradual loss of the liver' functions and the most common type of primary liver cancer in adults occurring in the setting of chronic liver inflammation. The causes of terminal liver diseases are persistent viral infections (such as Hepatitis B or C viruses), alcohol abuse and also obesity and type 2-diabetes.

### **ROLE OF GUT MICROBIOTA ON METABOLIC AND LIVER DISEASES**

The gut microbiota is an aggregate of microorganisms composed of bacteria, fungi, archaea and viruses. A healthy gut contains a balanced mixture of many commensal (beneficial) species. Under certain circumstances a non-physiological shift in this balance can promote development of microbes that are not beneficial anymore and capable to install or favor diseases.

Recently, it has been shown the gut microbiota is an important player in the pathophysiology of many intestinal and extra-intestinal diseases.

The liver is the organ which is in closest contact with the intestinal tract, and is exposed to a substantial amount of bacterial components and metabolites. Various liver disorders are known to be modulated by an altered microbiota.

Knowledge concerning the impact of microbiota composition and metabolic output on liver diseases remain largely unknown especially in this region.

### **THE PROJECT**

The scientists from Instituts Pasteur in Paris, in Morocco and in Iran work together in recent years on chronic liver diseases. In this project, they want to tackle the issue of the crosstalk between persistent infections in the liver, metabolic diseases and gut microbiota in MENA populations. To this aim, they will characterize the gut microbiota composition on a large series of patients with liver diseases originating from Morocco and Iran. In addition, they will study the host genes polymorphisms known to interact with gut microflora to assess their role in liver diseases. They will also evaluate the pro or anti-tumor compounds produced by the microbiota such as short-chain fatty acids (major players in innate immunity, metabolism and cell differentiation) in order to identify their impacts on the disease progress.

This project will increase our knowledge about the role of gut dysbiosis associated with liver diseases in order to set up prevention measures capable to thwart liver diseases progression.

\* This project also involves / involved the participation of external partner(s).

DEPARTMENT FOR SCIENTIFIC PROGRAMMING AND INCENTIVE ACTIONS - SPAIS



Liver cancer causes the death of 800,000 people each year in the world.

Liver Cancer and Cirrhosis are approximately responsible of 27,000 and 93,000 deaths per year in the MENA.



# Characterize structurally and functionally the centrosome components of Toxoplasma gondii, in order to identify potential novel therapeutic targets



### Project ACIP n° 76-2017

Scientific coordinator: Dr Maria FRANCIA, Molecular Biology Unit - Institut Pasteur in Montevideo Institut Pasteur International Network collaborators : Dr Mathieu GISSOT, Institut Pasteur in Lille (France) and Dr Philippe BASTIN, Institut Pasteur (Paris)

### WHAT ARE APICOMPLEXA AND TOXOPLASMA GONDII?

The **apicomplexa** are unicellular organisms and a class of protozoa. They comprise many pathogens responsible for important diseases in both animals and humans, including malaria, toxoplasmosis. Apicomplex are obligate intracellular pathogens, they must invade a host cell in order to replicate and survive.

**Toxoplasma gondii** is the etiologic agent of Toxoplasmosis. It is one of the most common parasitic infections of humans, with a worldwide distribution. Nearly one-third of humanity has been exposed to this parasite. Infection usually occurs by eating undercooked contaminated meat, exposure from infected cat feces, or mother-to-child transmission during pregnancy. In most adults, toxoplasmosis is asymptomatic, but it can cause blindness and mental retardation and death in congenitally infected children and devastating disease in immunocompromised individuals. The parasite completes its life cycle in cat's gastrointestinal tract and is present in the environment via the feces of the cat.

### **CENTROSOME AND CENTRIOLES**

The centrosome is an organelle located near the nucleus in the cytoplasm that divides and migrates to opposite poles of the cell during the replication process. The centrosome is composed of two parallel centrioles that are made up of nine sets of microtubule triplets arranged in a cylinder. The centrosome has a fundamental role in orchestrating central events to apicomplexan cell division, and flagellar assembly during sexual differentiation.

Recent findings suggest that apicomplexan centrioles are composed of a mixture of conserved and unique proteins. The latter remain largely unexplored and represent a possible rich source of drugable targets.

### **THE PROJECT**

Scientists from IP Montevideo, Lille and Paris associate their complementary expertise and propose a multidisciplinary approach focused on deciphering mechanistic underpinnings of intercellular replication, as a prominent source of pathogenesis, due to its fundamental role as describe above, using Toxoplasma gondii as a model. For this, they characterize the protein repertoire of the apicomplexan centrosome by generating an extensive interactome map of core structural proteins. Then, they will dissect the roles of identified proteins in the centriole biogenesis and homeostasis maintenance pathways by combining genetic manipulation and advanced microscopy.

This project will allow the identification of novel centrosomal proteins that could be essential for the survival of parasitic unicellular organisms. And therefore these novel centrosomal components will be potential novel therapeutic targets to fight apicomplexan diseases. 30–50% of the global population has been exposed to and may be chronically infected with T. gondii.

Each year, it is estimated that there are approximately 680 000 new cases of toxoplasmosis in France.

## Identify and characterize anti-malarial small molecules targeting the key *P. falciparum* protein (pfLDH) that will bring a novel concept for antimalarial therapies



### Project ACIP n°93-2017

Coordinator: Dr Azar TAHGHIGHI, Medicinal Chemistry laboratory - Institut Pasteur in Iran Institut Pasteur International Network collaborators: Dr Arnaud BLONDEL, Institut Pasteur (Paris), Dr Lawrence AYONG, Centre Pasteur du Cameroun

### MALARIA

Malaria is a potentially fatal parasitic disease caused by several species of parasites of the genus Plasmodium of which Plasmodium falciparum and Plasmodium vivax are the most dangerous.

P. falciparum is transmitted to humans by bites of infected female Anopheles mosquitoes. After the bite, the parasite infects liver cells where it multiplies. These new parasites are released into the blood stream, infect red blood cells and multiply. Red blood cells burst, initiating a cycle of red blood cells infection by series of parasite new generations.

The symptoms of malaria, including fever and cerebral malaria, are related to the blood stage of the infection.

### CURRENT TREATMENTS AND ITS LIMITS

Malaria is a public health tragedy and a major economic burden. Despite all the efforts and investments done over the last decade to roll back the disease, malaria is still responsible for detrimental clinical cases and deaths.

Anti-malarial drugs with guinoline scaffold have been used for malaria chemotherapy for a long time, which led to extensive spread of drug resistance. Clinical evidence for artemisinin resistance was first reported in a 2008 study leading to a recommendation for the use of combination therapy to prevent the apparition of resistance as much as possible. Development of parasite resistance to multiple anti-malarial drug classes has limited the use of several previously effective antimalarial drugs in most endemic countries worldwide.

Nowadays, there is still no effective vaccine on the market. The emergence of resistance even to the most recent combinations of anti-malarial drugs (ACTs) makes an urgent need for the development of novel anti-plasmodial compounds .

### THE PROJECT

The three teams involved in this project propose a multi-disciplinary approach involving bioinformatic, medicinal chemistry and biology to identify potent anti-malarial small molecules targeting the key P. falciparum protein, Lactate dehydrogenase (pfLDH). This protein is a key enzyme in malaria parasite and has been considered as a potential molecular target for anti-malarial drugs. Indeed the inhibition of enzyme blocks the metabolic pathway needed for the energy production of the parasite. Previously, molecular docking studies, in silico approaches used to predict how a protein (enzyme) interacts with small molecules (ligands), identified itraconazole, like potential inhibitor of pfLDH.

In this project, the scientists select, by docking and molecular dynamic studies, itraconazole analogues that are predicted to make the best interactions with the active pfLDH active site. The most promising compounds shall be chemically synthesized and structural characterized . Then, the in vitro anti-malarial activity of the synthesized compounds shall be tested. Compounds exhibiting potent anti-malarial activities will be evaluated in vivo for their cytotoxicity effects and for their efficacy in mouse malaria models.

The molecules thus identified should open a new way in the treatment against malaria.

In 2015, 212 million clinical cases and 429 000 deaths, most of them children living in sub-Saharan Africa where a child dies from malaria every two minutes

Itraconazole is an antifungal medication used to treat a number of fungal infections.

# Better understanding the transmission of Enteroviruses between animals and humans in order to reduce the risk of new emergence



### Project ACIP n° 162-2019

Scientific coordinator: Dr Maël BESSAUD, Viral Populations and Pathogenesis Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Ionela GOUANDJIKA-VASILACHE, Institut Pasteur in Bangui, Dr Serge-Alain SADEUH-MBA, Pasteur Centre in Cameroon \*

### WHAT ARE ENTEROVIRUSES?

Enteroviruses (EVs) are small viruses belonging to *Picornaviridae* that circulate worldwide. More than 300 types of EVs have been identified which fall into 15 species: EV-A to L and rhinovirus (RV) A to C. Viruses of species EV-A to C, some EV-Ds and RV-A to C mainly infect humans.

Except RVs and some EVs (such as EV-D68), which are respiratory viruses, EVs mainly replicate in the gut and are excreted in faeces. Transmission occurs through the fecal-oral route and is facilitated by low level of hygiene and sanitation.

Hundreds of EV types are known to infect humans and these infections are very common, especially during childhood.

Most EV infections are asymptomatic or trigger mild symptoms and resolve without sequelae. However, in some cases, severe complications can occur such as for polioviruses (members of species EV-C) which are the etiologic agents of poliomyelitis, a disease characterized by acute flaccid paralysis. In acute infection, EV can induce a wide range of severe manifestations such as neurological disorders, respiratory illnesses, neonatal sepsis-like disease, myocarditis that can lead to death. Chronic EV infection could also be associated with type I diabetes.

### TRANSMISSION OF EVS BETWEEN ANIMALS AND HUMANS?

Besides EVs that infect humans, some EVs were identified in other mammal species. As these animal EVs were genetically distinct compared to EVs found in humans, it has been believed for decades that EVs were quite specific to their respective host.

However, several observations suggest that EV transmission can occur between different species. In particular, some EV types that circulate in non-human primates have puzzling genetic links with human EVs. Among them, some were probably recently transmitted to humans by apes living in central Africa. The frequency of zoonotic transmission of EVs and the ways of transmission between animals and humans (in both directions) are completely unknown. In particular, the role herd animals could play as intermediates between wild animals and humans remains to be determined. The ability of animal EVs to establish sustained transmission from human to human and their pathogenic power on humans have also to be evaluated to determine whether wild and/or herd animals constitute niches from which new human EVs can emerge.

Millions of people are infected worldwide on an annual basis, especially infants and children under the age of one.

Enterovirus 71 (EV-A71) is a virus type commonly associated with severe neurological complications during hand, foot, and mouth disease outbreaks, particularly in South-eastern Asia.

This project involves three teams of the Institut Pasteur International Network (Central African Republic, Cameroon and Paris) with significant expertise in polioviruses and non polio enteroviruses.

This project constitutes a pioneering work that aim to characterize EVs that circulate among domestic and wild animals in central Africa, a geographical region that harbours a highly diverse wild fauna, where close contact between humans and herd animals are still common and where poor sanitation systems provide a conducive environment for EV transmission. They will be able to determine whether EVs are transmitted between herd animals and humans, in both directions. This work will also help in determining whether genetic exchanges between animal and human EVs occur to give rise to new viruses harbouring chimeric genomes and whether such viruses actively circulate among humans. Besides, new deep sequencing methods and reverse genetics will be use to study original viruses that were detected a decade ago in stool samples of wild apes living in the Cameroonian rainforest.

This project will give new insights about the zoonotic transmission of EVs in developing countries and will help in identifying potential sources of emergence of new EV types and/or new EVs genes in humans. These data will enable health authorities in these countries to implement preventive measures to prevent new emergence.

Recently, enterovirus D68 (EV-D68) emerged as the causative agent of sporadic but severe respiratory disease outbreaks across the United States, Asia, Africa, and Europe.

# Better understanding the Dengue virusmosquito-host interactions in order to develop effective antivirals



### Project ACIP n° 221-2019

Scientific coordinator: Dr Carolina SCAGNOLARI, Laboratory of Virology - Institut Pasteur - Cenci Bolognetti Foundation (Rome, Italy)

Institut Pasteur International Network collaborators: Dr Tineke CANTAERT, Institut Pasteur in Cambodia, Dr Darragh DUFFY, Institut Pasteur (Paris), Dr Zanakoungo Ibrahima COULIBALY, Institut Pasteur in Ivory Coast \*

### WHAT IS DENGUE?

Dengue is an infectious disease caused by Dengue virus (DENV), of the family *Flaviviridae* and is the most prevalent mosquito-borne disease. The virus is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes DENV is endemic in tropical and subtropical regions throughout the world and are ubiquitous in both rural and urban settings.

Currently, 4 serotypes of dengue virus are recognized and the co-circulation of multiple dengue serotypes in the same area has been documented.

DENV infection affects infants, young children and adults. This infection causes a wide variety of clinical manifestations that range from asymptomatic to severe dengue fever (DF), that in some cases may evolve to the more severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The most significant risk factor for the development of DHF/DSS is acquisition of a second infection with different serotype.

There are currently no therapeutics or antiviral drugs for this disease and the only DENV vaccine available has major restrictions in usage and a low efficacy against certain DENV serotypes.

### HUMAN IMMUNE SYSTEM AND DENGUE VIRUS

The lack of availability of drugs or efficient vaccines can be mainly attributed to an incomplete understanding of complex dengue immunopathogenesis. It is extremely important from public health point of view to understand how the early immune system responds to infection and how the virus evades immune responses in order to develop effective antivirals and ameliorate vaccines. The activation of the antiviral innate defense system at the early phase of DENV infection appears to play an important role in the control of virus replication. However, it is currently unknown whether changes in the innate immune response have beneficial or deleterious effects in DENV infection and replication according to the progression of disease. Intriguingly, persistent DENV propagation in mosquito tissues neither results in dramatic pathological sequelae nor impairs the vector behavior or lifespan, indicating that mosquitoes have evolved mechanisms to tolerate persistent DENV infection.

Nevertheless, recent studies revealed that mosquito saliva alone has also profound effects on the human immune system.

The biological significance of these changes remains to be determined, but it might explain how DENV generates in humans an early immune environment more permissive to the establishment of infection.

DENV is endemic in more than 120 countries around the world.

Over half of the global population is at risk for DENV infection.

The scientists from four institutes of Institut Pasteur International Network (Rome, Paris, Cambodia and Ivory Coast) will combine their expertise in virology, immunology and entomology to perform a comprehensive characterization of mechanisms underlying the interaction between DENV infection, mosquito vectors, and the early host antiviral immunity. Through a comprehensive evaluation of distinct molecular and cellular determinants of the antiviral immunity in the blood samples collected from a selected cohort of hospitalized, acute infected Cambodian children, who experienced mild and severe illness following secondary DENV infection, they will be able to unveil the association of immunological and physiological pathways that underlie complex DENV illness. For that, they will use ultrasensitive innovative technologies to allow an accurate measure of the magnitude of innate immune response. Moreover, the analysis of the capability of mosquitoes' salivary gland homogenates to manipulate the host's early immune response, will permit to study mosquito-host interactions.

This project will contribute to (i) a better understanding of the viral and host factors that determine the pathogenesis and outcome DENV infection and also (ii) open new important avenues for the identification of human and/or mosquito therapeutic targets for a more efficient managment of DENV disease.

Dengue affects approximately 400 million people annually, with 100 million symptomatic cases.

Development of a new genetic tool to reduce the vectorial capacity of *Anopheles* mosquitoes in order to fight against malaria



### Project ACIP n° 253-2019

Scientific coordinator: Dr Abbasali RAZ, Malaria and Vector Research Group - Institut Pasteur in Iran Institut Pasteur International Network collaborators: Dr Mathilde GENDRIN, Institut Pasteur in French Guiana, Dr Christian MITRI, Institut Pasteur (Paris) \*

### WHAT IS MALARIA?

Malaria is a serious, life-threatening parasitic diseases in humans, caused by five different members of the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*).

*Plasmodium* is transmitted to humans through the bites of infected female *Anopheles* mosquito. About 30 species of *Anopheles* are responsible for transmitting of malaria parasite species.

The most common symptoms of malaria are headaches, fatigue, low energy, nausea and vomiting. The symptoms of malaria which are related to the blood stage of infection by the parasite are fever and cerebral malaria.

Currently, several antimalarial drugs could be used (1) for prophylaxis but do not guarantee absolute protection against infection or (2) for therapeutics decreasing only the duration and severity of the disease. One of the obstacles in fighting against malaria is the resistance of parasite to current drugs.

The main way to prevent and reduce the transmission of malaria is vector control (spraying insecticides inside houses, insecticide treated bed nets). In addition, the control of vectors is hampered by the emergence of mosquito resistance to insecticides.

### **REDUCE THE VECTORIAL CAPACITY OF MOSQUITOES**

For decades, different approaches have been designed and used to control and reduce the burden of malaria which some of them are based on targeting the vectors. One of the strategies to limit the role of mosquitoes in disease transmission is paratransgenesis. This involves making some genetic modifications in the symbiotic microorganisms of the mosquito to reduce its vectorial capacity. Furthermore, it has been revealed that RNAi technology is an efficient method for inactivation or suppression of the targeted gene in insects by double stranded RNA (dsRNA) molecules. Delivery of dsRNA into the target cells is one of the main obstacles to reach to successful silencing in mosquitoes in field situation.

The *Asaia* bacterium is frequently found in the microbiota of multiple *Anopheles* mosquitoes including major malaria vectors in Africa, Asia and America. *Asaia* spp. are efficient for dsRNA production and delivery to different insect species. This bacteria have two specific characters that differentiate them from other symbionts which are transovarial and transstadial transmission therefore those could spread between populations and different generations of mosquitoes.

It has been confirmed that RNase III (*rnc*) mutant bacteria (enzyme that recognizes and degrades dsRNA) are more efficient to produce and release more dsRNA molecules.

All these aspects make *Asaia* as a vigorous and robust symbiotic nominee for using in paratransgenesis and combatting against malaria.

\* This project also involves / involved the participation of external partner(s).

Annually, number of reported clinical malaria cases is approximately 216 million causing almost 0.45 million deaths every year.

The main groups at risk are children under the age of 5 years, pregnant women and non-immune adults.

Scientists from Institut Pasteur in Iran, French Guiana and Paris will combine their complementary expertise to develop the tools using *Asaia* spp. in order to produce and delivery double stranded RNA against two specific genes into the *Anopheles* mosquito vectors to reduce their vectorial capacity. For that, they will characterize the *rnc* gene in *Asaia* spp. and they will create an *rnc* mutant strain. They will engineer this strain to produce dsRNA in *Anopheles* body against two important genes (*semaphorin* and *Miso*) for inhibiting the mosquito development and affecting reproductive success respectively. Finally, using these engineered strains, they will evaluate the efficacy of this approach in mosquitoes in adult and larval mosquitoes as a potential tool to reduce vectorial capacity.

This study will pave the way towards more efficient gene silencing in adult and larval mosquitoes, which will be an interesting genetic tool as well as a novel paratransgenesis approach.

# Develop a new approach for diagnosis of viral infection in mosquitoes in order to detect pathogen emergence automatically in the field and prevent outbreaks



### Project ACIP n° 255-2019

Scientific coordinator: Dr Jean-Bernard DUCHEMIN, Medical Entomology Unit - Institut Pasteur in French Guiana Institut Pasteur International Network collaborators: Dr Christian VESTERGAARD and Albane IMBERT, Institut Pasteur (Paris), Dr Rabiou LABBO, CERMES (Niamey, Niger)

### WHAT ARE MOSQUITO-BORNE DISEASES?

Mosquito-borne diseases are diseases caused by bacteria, viruses, or parasites transmitted by bites of an infected mosquito. Some viruses are called arbovirus such as dengue, chikungunya, yellow fever virus.

Because of these microbes, mosquitoes are one of the deadliest animals in the world.

Mosquitoes transmit diseases to both humans and animals. The main human mosquito-borne diseases include malaria, West Nile fever, yellow fever, dengue, chikungunya, and Zika.

Symptoms of illness are not always specific to the type of viral infection and vary on severity, based on the individuals infected.

In the last ten years, Zika, Dengue and Chikungunya viruses have reached new areas of distribution and produced a huge burden in human health. Despite having largely been considered historical and doomed by a very effective vaccine, Yellow Fever virus (YFV) have triggered outbreaks in Brazil and Angola with several thousands of cases, these last years.

Prevention and control of these diseases are based on effective measures for vector control and individual protection against mosquito bites.

### PREVENT AND LIMIT ARBOVIRUS CLINICAL CASES

In most cases, vector control is the only method at hand. Many emerging or reemerging arboviruses are of zoonotic origin, and the knowledge of patterns of viral circulation within animal populations and between human and animal communities is primordial for mitigating emergence.

Currently, the diagnosis for virus circulation needs large surveys of sentinel animals and/or mosquito trapping. These deployments involve highly skilled human resources and time-consuming procedures, making it difficult for them to be highly reactive.

Automation of surveys via autonomous traps would improve the reactivity of the whole system and allow quicker and more adapted responses.

In addition, several examples indicate that arbovirus infection may trigger behavioral change in mosquitoes. These changes may be virus specific, and for example, differ between the two flaviviruses dengue and zika. However, a large panel of mosquito behaviors is poorly known and quantified.

About 500 million cases of mosquito-borne diseases occur annually.

Mosquito bites result in over 1 million deaths every year. The majority of these deaths are due to mosquito-borne diseases, most prominently malaria.

Scientists from three institutes of the Institut Pasteur International Network (French Guiana, Paris and Niger) will combine their complementary expertise to develop a new approach for the diagnosis of viral infection in mosquitoes based on the analysis of their behavior. The present project aims to demonstrate that the statistical signature of behavioural changes in mosquitoes after YFV infection can be leveraged to detect the virus circulation in transmission areas. To establish this, the behaviour of mosquitoes will be recorded (using a high definition camera) within small arenas designed for analysis of different behaviours such as flight, and orientation.

After the baseline behaviour of uninfected mosquitoes are established, monitoring of mosquitoes experimentally infected with the YFV will be implemented, and data analysed using supervised learning to identify the best proxy for infection diagnosis. At the same time, behaviour platforms will be fitted to standard traps to record and analyse the diversity of wild mosquito activity and behaviour in the field.

This will not only help to improve epidemiological models, but may furthermore pave the way to design low-cost detectors to survey circulating viruses and detect pathogen emergence automatically in the field. Sustained mosquito control efforts are important to prevent outbreaks from mosquito-borne diseases.

# Provide insights to the epidemiologic and genetic characteristics of EV-D68 in two sub-Saharan African countries in order to prevent new outbreaks.



### Project ACIP n° 275-2019

Scientific coordinator: Dr Ndongo DIA, Respiratory Viruses Research Group - Institut Pasteur in Dakar Institut Pasteur International Network collaborators: Dr Marius ADAGBA, Institut Pasteur in Ivory Coast, Dr Maël BESSAUD, Institut Pasteur (Paris) \*

### WHAT IS ENTEROVIRUS 68?

Enterovirus D68 (EV-D68) is a re-emerging pathogen which was first isolated in 1962 from children with pneumonia and bronchiolitis. The virus is a member of enterovirus D species (EV-D), which belongs to the genus *Enterovirus* and the family of *Picornaviridae*.

EV-D68 can causes respiratory illness with mild to severe symptoms or no symptoms at all. Mild symptoms include runny nose, sneezing, cough, body and muscles aches. Severe symptoms result in wheezing and difficulty breathing.

The virus is transmitted through close contact with an infected person, or by touching contaminated objects or surfaces. The virus can be found in saliva, nasal mucus or sputum from infected person.

Infants and children are the most at risk for this virus. However, adults with weak immune systems and those with severe chronic medical conditions have also a big risk for severe complications.

EV-D68 can only be diagnosed by doing specific lab tests on specimens from a person's nose and throat, or blood.

Currently, there is no specific treatment and no vaccine to prevent EV-D68 infection.

### **ENTEROVIRUS 68 IN AFRICA**

Until 2008, EV-D68 was rarely reported. Between 2008 and 2014, small outbreaks of EV-D68 respiratory disease were observed worldwide. In 2014, the first large outbreak of EV-D68 was reported in North America associated with considerable morbidity and mortality. Clinical manifestations were mainly respiratory distress, tachypnea, hypoxaemia, wheeze, and chest pain. Notably, unexpectedly, this outbreak of severe respiratory infections coincided with an upsurge of polio-like neurological symptoms. The paralysis seen in patients infected with EV-D68 was clinically defined as acute flaccid myelitis (AFM). Consequently, surveillance studies for EV-D68 were launched worldwide which resulted into increased detection and enhanced data collection. In many areas, local clusters of EV-D68 infections were repeatedly reported, including cases of severe respiratory disease and AFM. However, in Africa, there is few published data on the clinical and epidemiological features of EV-D68 infection, and no active public health surveillance is implemented for this virus.

Recent retrospective studies from Senegal highlighted the circulation of EV-D68 in Influenza-like illness patients during both outbreaks, hence the need to pay special attention to the EV-68 in sub-Saharan African countries.

Enteroviruses are very common viruses. There are more than 100 types of enteroviruses including EV-D68.

EV-D68 has been associated almost exclusively with respiratory disease. Though EV-D68 usually causes mild to severe respiratory illness, the full spectrum of EV-D68 illness is not well-defined.

Three Institutes of Institut Pasteur International Network (Dakar, Ivory Coast and Paris) involved in this project will combine their complementary expertise to investigate the burden and clinical presentations of EV-68 infections in pediatrics facilities in Senegal and Ivory Coast and to provide insights to the epidemiologic and genetic characteristics of EV-D68 in both countries. For that, they will focus on children with acute respiratory infections or with AFM or any other neurological syndromes with paralysis. They will detect EV-D68 by a specific real-time RT-PCR from respiratory tract samples and stools then they will isolate viruses. Finally they will perform complete genome sequencing and phylogenetic studies of these viruses in order to compare the EV-D68 strains that circulate in West Africa with those found elsewhere.

These prospective study will provide new insights in the role of EV-D68 regarding severe respiratory infection and acute flaccid myelitis and understanding the evolutionary aspects of EV68 strains isolated in both countries.

Prompt diagnosis and continued surveillance of EV-D68 infections are essential to managing and preventing new outbreaks.

# A better understanding of the determinants of the emergence of dengue virus serotypes in order to better document risk assessment



### Project ACIP n° 281-2019

Scientific coordinators: Dr Myrielle DUPONT-ROUZEYROL and Dr Catherine INIZAN, Dengue and Arboviruses Expertise and Research Unit - Institut Pasteur in New Caledonia

**Institut Pasteur International Network collaborators:** Dr Etienne SIMON-LORIERE and Dr Arnaud TARANTOLA, Institut Pasteur (Paris) and Dr Tineke CANTAERT, Institut Pasteur in Cambodia \*

### WHAT IS DENGUE?

Dengue infection is caused by dengue virus (DENV), an arbovirus (arthropodborne virus) transmitted to humans by the bite of infected mosquitoes, mainly from the genus *Aedes*.

Dengue has become the most prevalent human arbovirosis, affecting mainly tropical and subtropical countries albeit recently expanding to more temperate countries.

The spectrum of dengue clinical presentations is broad, ranging from asymptomatic to severe, sometimes fatal, infections. Symptomatic dengue is a self-limiting illness characterized by an inconstant combination of symptoms lasting for 2-7 days: fever, nausea, vomiting, rash, severe headache, pain behind the eyes, muscle and joint pains, swollen glands. Some cases however, present warning signs (abdominal pain, persistent vomiting, clinical fluid accumulation, mucosal bleeding, ...) and can progress to severe dengue, characterized by severe plasma leakage leading to shock and/or fluid accumulation with respiratory distress, severe bleeding and/or severe organ involvement.

To date, there is no specific dengue therapeutics. A vaccine is available but with major restrinctions in usage. Prevention is currently limited to vector control measures, which regularly fail at preventing dengue outbreaks.

### IMPACT OF DENGUE SEROTYPES REPLACEMENTS ON DENGUE EPIDEMIOLOGY

DENV are divided into 4 serotypes, DENV-1 to 4. Infection with any of these serotypes is thought to provide lifelong immunity towards reinfection with the same serotype. In dengue-endemic countries, the 4 serotypes usually co-circulate. In countries where dengue has an epidemic profile of circulation, as in New Caledonia, serotypes cause successive outbreaks involving one serotype at a time. A minority serotype may, however, co-circulate along with the majority serotype. This minority serotype can either vanish or emerge as the majority serotype upon the next epidemic season.

Emergence of a new serotype in a partially naïve population, and to some extent immune to the previous major serotype, can potentially lead to a large and severe outbreak. Evaluating the epidemic potential of a minority serotype is therefore of prime importance to forecast outbreaks and provide more accurate risk assessments. Drivers of the emergence of minority serotypes are not yet identified. New Caledonia is an ideal observatory of the drivers of the emergence of minority serotypes because of its "simple" ecosystem. WHO estimates that 3.9 billion people, in 128 countries, are at risk of infection with dengue virus.

Approximately 390 million dengue infections each year worldwide.

The present project, which associates scientists from Institut Pasteur of New Caledonia, Paris and Cambodia, aims to identify the determinants of the emergence of minority serotypes during dengue outbreaks. The respective roles of viral and human factors in the emergence of a new serotype will be explored. First, genetic evolution of minority serotypes will be characterized as per their emergence or extinction using serum samples from patients infected by minority serotypes. In parallel, viral fitness of emerging and non-emerging minority serotypes will be compared *in vitro* in human cells. Second, the impact of immunity to the majority serotype on the emergence or extinction of a minority serotype will be evaluated using serum samples from persons immune to the previous majority serotype.

Overall, this project will provide an extensive and integrated characterization of viral and human factors impacting the natural cycle of circulation of minority serotypes, driving their emergence or extinction. The accurate evaluation of the epidemic potential of a minority serotype would be a highly valuable parameter to forecast the occurrence of massive dengue outbreaks. A better understanding of the determinants of the emergence of DENV serotypes is crucial for improved preparedness in populations, health authorities and clinicians. In New Caledonia, already 3,900 dengue confirmed cases have been notified by the Health Authorities since January 2019, and two deaths have been recorded.

# Understand how cellular nucleases play a crucial role in the inhibition of HBV infection to develop a new therapeutic strategy



### Project ACIP n° 318-2020

Scientific coordinator: Dr Jean-Pierre VARTANIAN, Molecular Retrovirology Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Yu WEI, Institut Pasteur of Shanghai - Chinese Academy of Science, Dr Siriphone VIRACHITH, Institut Pasteur in Laos \*

### WHAT IS HEPATITIS B?

Hepatitis B is an infection of liver caused by the Hepatitis B Virus (HBV). It is a major global health problem.

Most newly infected individuals show no symptoms. However, some people have an acute condition, with symptoms that persist for several weeks. In some people, the hepatitis B virus can also cause a chronic infection of the liver, which may later develop into cirrhosis or liver cancer.

The virus is most commonly transmitted from mother to child during birth and delivery, as well as through contact with blood or other body fluids. It infects only hepatocytes in the liver and its life cycle takes places in both cytoplasm and nucleus of these infected cells.

Current antiviral treatments can effectively inhibit HBV replication, but unfortunately they do not allow total elimination of the virus. Indeed, after discontinuation of treatment, a rebound in viral load with an exacerbation of hepatic disease is observed, thus prolonged administration of these drugs is required for a majority of patients. This long-term antiviral treatment with nucleotide/nucleoside analogs can be also accompanied by the emergence of viral resistant strains. Thus, it appears urgent to develop new antiviral strategies.

### **HOW TO ERADICATE THE VIRUS?**

The viral persistence is due to the maintenance of HBV covalently closed circular DNA (cccDNA) in the nucleus of infected cells and deregulation of the host's innate and adaptive immune response. Currently, while there are many antiviral treatments that effectively inhibit HBV replication, these treatments do not target HBV cccDNA at the nuclear level in infected hepatocytes, they are thus not curative.

Previous works by coordinator's team suggests that expressed cellular nuclease, DNase I, in hepatocytes at low  $O_2$  environment (physiological conditions *in vivo*) will be encapsidated in HBV and induce the degradation of neosynthesized relaxed circular (RC)-DNA. The formation of nucleocapsids containing these nucleases will be recycled to the nucleus and degrade HBV cccDNA as well as nuclear DNA leading infected cells to apoptosis.

In contrast to current antiviral treatment that only inhibits viral DNA synthesis, cellular nucleases may have anti-HBV potentials that lead to eradication of viral-infected cells.

More than 370 million people live with chronic hepatitis B infection worldwide and over 600,000 annual deaths.

HBV infection is a major public health worldwide, particularly in Asia. Indeed in Lao People's Democratic Republic (PDR), infections during childhood often are not cleared and result in chronic HBV, which can manifest later as hepatocellular carcinoma, with high morbidity and mortality. Indeed, Lao PDR has one of the highest rates of liver cancer in the world.

The scientists from three institutes of Institut Pasteur International Network (Paris, Shanghai and Laos) will combine their expertise to understand how hypoxic environment and host cellular restriction factors work hand in hand to annihilate HBV infection. For that, they will demonstrate that the presence of encapsidated DNase I will degrade nascent viral genomic DNA and will lead HBV nucleocapsid containing nucleases to degrade HBV cccDNA as well nuclear DNA by releasing the nucleases into the nucleus of infected cell lines.

Then, they will validate these *in vitro* obtained data, in mouse models and to prove *in vivo* the functionality of the nucleases in the treatment of persistent HBV infection. They will screen for the cellular nucleases in HBV chronic infected patients in order to investigate the relationship between the nucleases and clinical parameters such as viral load, liver function, stage of disease and HBV genotype in HBV chronically infected individuals.

This project will pave the way for the development of a new therapeutic strategy for the treatment of chronic hepatitis B.

# Understand why people with Rheumatoid Arthritis have the flu more often and how to help them



### Project ACIP n° 328-2020

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**Institut Pasteur International Network collaborators:** Dr Milena HASAN (UTechS Cytometry and Biomarkers), Institut Pasteur (Paris) and Dr Cyril BARBEZANGE, Sciensano (Brussels, Belgium)

### WHAT IS RHEUMATOID ARTHRITIS?

Rheumatoid arthritis (RA) is a progressive auto-immune joint inflammatory disease. The disease probably starts because of a combination of genetic, biological and environmental factors, in particular smoking.

It causes inflammation of several joints at once, which swell, become painful, and are limited in their range of motion. Over time and without treatment, it leads to bone destruction and joint deformity, increasingly disabling the patients and limiting their physical capacity and every-day activities. RA has substantial negative effects on the health-related quality of life of patients, which are not limited to the physical components of well-being. RA is associated with a high prevalence of comorbidities such as depression, pulmonary disease, cardiovascular disease, and infections of bacterial and viral origin.

There is currently no treatment that can cure rheumatoid arthritis. Current treatments are only aimed at slowing the progression of the disease and providing effective relief to patients. These treatments can be immunosuppressants or inhibitors of inflammation.

### RHEUMATOID ARTHRISTIS AND INFLUENZA INFECTION

One study with arthritic patients found a nearly 3-fold increase in the incidence of Influenza complications (mostly pneumonia and stroke/myocardial infarction) in RA-patients vs. sex- and age-matched controls. However, the incidence of Influenza infection and its complications was increased to a similar extent in patients on therapy, as in those who were not. This suggests that the state of chronic inflammation, characteristic of RA, might contribute to the increase in the patients' susceptibility to the flu. Why?

In recent years there has been accumulating evidence that the joint inflammatory milieu in rheumatoid arthritis affects the epigenetic machineries of various cell types involved in its pathogenesis. When an individual with RA is infected with the influenza virus does this virus also play a role in changes to host mRNAs?

Furthermore, while epigenomic studies on influenza-infected laboratory animals are scarce, *in vivo* studies are completely lacking when it comes to the host (epi) transcriptome. This is especially true when it comes to double-hit inflammation models, such as RA complicated by an influenza infection. Finally, it remains unclear what influenza virus strain dependent differences, if any, exist (e.g. seasonal vs. pandemic flu strains) in terms of their effect on the host (epi)transcriptome.

Approximately 1% of the adult population worldwide has RA, with a reported disease incidence of 20-50 new cases per 100 000 annually in Europe and North America alone.

It is two to three times more common in women than in men.

Scientists from 3 institutes of Institut Pasteur International Network (Sofia, Paris and Brussels) will combine their expertise, spread across the fields of immunology, epigenetics, and infection biology to assess whether the epigenetic code of transcripts and the genomic organization of lung epithelial cells is modified by rheumatoid arthritis (RA) and influenza infection, and whether RA thereby increases the susceptibility to infection.

By combining classical approaches with technological innovations, they will investigate host lung epithelium gene expression and the mRNA m6A modification, as well as chromatin accessibility in individual lung epithelial cells from arthritic Balb/c mice infected with a seasonal influenza virus H3N2. They will study an *in vitro* model of the human airway epithelium, whether or not putative changes in the host (epi)transcriptome are strain specific and / or dependent on the inflammatory environment.

This project will bring knowledge at the fundamental level but also help in the prediction and identification of the RA patients who are most vulnerable to the flu and its more severe complications.

## Increasead understanding of how mutations cause polymyxin resistance to help develop molecular methods for detecting resistant isolates and new strategies to fight infections



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### WHAT ARE POLYMYXINS?

Polymyxins (PM) are antibiotics used in the treatment of Gram-negative bacterial infections. Due to adverse side effects (neurotoxic and nephrotoxic), their use was practically abandoned in humans.

PMs were originally isolated from soil bacteria, which use these cyclic positivelly charged peptides with a lipid tail, as a weapon of warfare to compete with neighbouring bacteria. A few structurally related groups of PMs exist, with colistin and polymyxin B being available for clinical use.

Polymyxins act as detergents, inserting themselves into the phospholipids of the bacterial membrane. The consequent disruption in permeability leads to leakage of cytoplasmic content.

With the global dissemination of carbapenem resistant lineages and the lack of new antimicrobials, PMs are in use again as last resort antibiotics to treat people infected with multi drug resistant or extremelly drug resistant bacteria, especially in hospital settings.

### **RESISTANCE TO POLYMYXINS**

Because the use of PMs has increased, resistant bacteria are being detected all over the world, even in healthy individuals. Indeed, it is alarming to find that colonization by PM resistant *Klebsiella pneumoniae* (PM-R *KP*) has been increasingly detected not only in hospital settings but also in communities of healthy individuals.

The main mechanism that bacteria use to become resistant is mutation (changes) in a few proteins (PmrAB, PhoPQ, and MgrB), which make the cell membrane less attractive to the positively charged antibiotic, thus reducing its efficiency to kill. PmrAB and PhoPQ are bacterial proteins that do "molecular talking" by sensing signals in the environment and triggering molecular signal relays that respond to this. The mutations that make bacteria resistant to polymyxins make bacteria become "chatterboxes" that continuously shape their cell membranes to resist them. But how do these mutations do this?

Though dozens or even hundreds of mutations have been described in PhoPQ and PmrAB of enterobacterial clinical isolates worldwide, the molecular basis of resistance is unknown.

Antibiotic resistance is one of the biggest public health challenges of our time.

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Antibiotic resistance can affect anyone, of any age, in any country.

The consortium will gather scientists from 3 institutes of Institut Pasteur International Network, with expertise on structural biology, biochemistry and antibiotic resistance, to discover how mutations in PhoPQ and PmrAB cause PM resistance. For that, they will identify mutations in these proteins with a causative role in polymyxin resistance in PM-R-KP isolates. They will produce and purify the mutant proteins in order to look at their structure (crystallography, NMR, SAXS) and biochemical properties, to reveal the molecular mechanisms of resistance.

This project will provide precise information on mutations that induce resistance, which will be very important to start developing molecular methods to detect resistant isolates.

The number of infections caused by antimicrobial resistant bacteria is on the rise and there are estimates that by 2050 more people will die of infections by resistant bacteria than due to cancer.

# Unraveling the roles of flea vectors to better understand the maintenance and emergence of bubonic plague in Madagascar



### Project ACIP n°363-2020

Scientific coordinator: Dr Mireille HARIMALALA, Medical Entomology Unit - Institut Pasteur de Madagascar Institut Pasteur International Network collaborators: Dr Javier PIZARRO-CERDA, Institut Pasteur (Paris), Dr Florent SEBBANE, Institut Pasteur in Lille

### WHAT IS PLAGUE?

Plague is a bacterial zoonosis caused by *Yersinia pestis*, which usually infects small mammals (e.g. rodents) and their fleas. It is transmitted from animals to humans through the bites of infected fleas, by direct contact with animal fluids, and more rarely, by ingestion of infected raw animal products. It can also be transmitted between humans or between domestic animals and humans by inhalation of aerosols. Depending on the route of infection, the disease occurs in three forms:

- -The bubonic form results from the bite of infected flea. The patients develop fever, headache, chills, weakness and painful lymph nodes (buboes).
- -The pneumonic form is due to human-to-human transmission or untreated bubonic form. The patients still present fever, headache and weakness but also display chest pain, coughs and blood-striped sputum.
- -The septicemic form is due to untreated bubonic plague, infected animal handling or infected flea bite. The patients develop fever, chills, abdominal pain and extreme weakness.

Today, plague remains endemic in Africa (Madagascar, Democratic Republic of Congo), Asia (China, Mongolia) and the Americas (Peru, USA) and it is currently considered as a re-emerging disease.

If the diagnosis is performed early, bubonic plague can be successfully treated with antibiotics (streptomycin, ciprofloxacin, gentamycin). Pulmonary plague is one of the deadliest infectious diseases, as patients may die 24 hours after infection.

### **HOW TO FIGHT PLAGUE?**

As human plague is a flea-borne disease, fighting it requires targeting its insect vectors that are the main responsible of disease transmission and spread. Little information is available especially on endemic fleas and reservoirs involved in *Y. pestis* maintenance in Madagascar. The scientists from IP Madagascar have observed that the endemic flea species *Synopsyllus fonquerniei* can be naturally infected by *Y. pestis* in forest areas. For such particular biotopes, it is therefore important to identify wild animals (fleas and their hosts) involved in plague maintenance, and to confirm whether they can get in contact with domestic animals (rats and rat fleas), resulting in human outbreaks. The characterization and comparison of *Y. pestis* strains circulating on those wild and domestic animals is also necessary to provide evidence of plague circulation between both environments.

Nearly 50,000 human cases of plague were reported to WHO by 26 countries between 1990 to 2015.

Scientists from three institutes from the Institut Pasteur International Network (Madagascar, Paris and Lille) with expertise in the field of plague, and collaborating within the framework of an Institut Pasteur International Unit, plan to investigate the involvement and the role of fleas in sylvatic plague maintenance and in human plague reappearance/re-emergence in Madagascar. To this end, they will sample flea species and their associated hosts that may be infected by *Y. pestis* in different sites and this, quarterly in a year. Three chosen areas of sampling are forest regions that have experienced/experience plague. The scientists will then isolate and characterize *Y. pestis* strains on fleas from wild and human environments. Finally, they will measure the vector competence of identified Malagasy flea populations in the lab, in order to understand whether it varies and whether it is associated with the heterogeneity of plague presence in the country.

The project will provide a better understanding of plague maintenance, emergence and transmission by flea vectors in Madagascar, which should help to better control the risk of infection in human populations.

Currently, Madagascar is the most affected country in the world with 300 to 600 cases reported every year.

The bubonic form represents the majority (86%) of reported cases in the country.

# Vitamin C : a novel diarrhea risk-factor?



### Project ACIP n°399-2020

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### WHAT ARE DIARRHEAL DISEASES?

Diarrheal diseases are a collection of diseases caused by multiple viral, bacterial, and parasitic organisms that share the common symptom of diarrhea, defined as the passage of three or more loose or liquid stools per day. Diarrheal diseases affect people of all ages throughout the world. Children, however, are the most vulnerable.

Septic bacterial infections and severe dehydration and fluid loss are the main causes of death from diarrheal diseases.

Infections are spread through contaminated food or drinking-water, or from personto-person as a result of poor hygiene.

Diarrheal diseases are generally managed by oral or intravenous rehydration therapy. However, only 33% of children in developing countries currently receive standard oral rehydration salt therapies to treat diarrheal episodes.

Diarrhea is a leading cause of malnutrition in children under five years old. Conversely, nutritional deficiency, especially vitamin-deficiency, may constitute a risk factor for diarrhea susceptibility.

### **RELATIONSHIP BETWEEN VITAMIN C AND DIARRHEAL DISEASES?**

Historically, the association of scurvy, induced by severe Vitamin C (Vit.C) deficiency, and diarrhea was suggested since the 18th century in populations suffering from malnutrition (ie. soldiers, seamen). Diarrhea was considered as a "symptom" or "part" of scurvy. Importantly, Vit.C supplementation allowed the treatment of both scurvy symptoms and diarrhea. Since these preliminary observations, if severe Vit.C deficiency has been confirmed as the main cause of scurvy, no clinical symptom or diseases have been associated with moderate Vit.C deficiency.

Vitamin C deficiency has never been defined as a risk factor for diarrhea. No reliable data are available regarding the plasma Vit.C level in children living in diarrhea endemic countries.

In addition, plasma Vit.C level is significantly decreased in septic patients. Many studies demonstrate the beneficial effect of Vit.C in response to LPS injury.

One of external partners involved in this project has been studying for several years the impact of Vit.C deficiency on the host response to bacterial infections, using bacillary dysentery (caused by *Shigella* spp., pathogenic enterobacteria) as a model. These studies demonstrated that moderate Vit.C deficiency increases the susceptibility of guinea pigs to *Shigella* spp. infection, associated with more severe and prolonged diarrheal episodes.

Vit.C seems to play a role in the susceptibility to enteric infections. However, this hypothesis has never been evaluated so far in a clinical study.

Diarrheal disease is the second leading cause of death in children although it is both preventable and treatable.

There are nearly 1,700 million cases of childhood diarrheal diseases every year.

Scientists from three institutes from Institut Pasteur International Network (Paris, French Guiana and Bangui) will combine their expertise to analyze the impact of Vit.C seric levels on susceptibility to enteric infections and diarrhea in children in countries with a high diarrheal burden. Furthermore, this project will provide a comprehensive picture of the distribution of Vit.C levels on children (2-5 years) in these countries. This project will take advantage of robust, long-term established collaboration between differents partners of this consortium. The pilot case-control study will be conducted in France (control), in Africa (Central African Republic) and in South America (French Guiana). For that, they will perform an epidemiologial study in these three countries involving children suffering of diarrhea compared to healthy ones. Then they will define standard plasma Vit. C values in these various geographic areas as well as the Vit. C levels in children suffering diarrhea. Finally, they will use these results to describe a possible increased risk of enteric infections associated with Vit.C deficiency.

This work will have an impact on the diarrheal disease prevention strategy and nutritional recommendations for children, but it will also allow the validation of animal models of infection deficient in Vit.C, which are still lacking for the main pathogens (*Shigella* spp., *Vibrio cholerae*,...).

Diarrhea kills around 800 000 children under five every year.

# Demonstrating persitence of immunity after abridged intradermal rabies post exposure prophylaxis to increase access to vaccination for the most vulnerable populations.



### Project ACIP n°403-2020

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### RABIES

Rabies is a viral disease that causes an acute inflammation of the brain in humans and other mammals. The rabies virus (RABV) is overwhelmingly transmitted to humans after the bite of an infected dog. All bites from an infected dog are not followed by RABV transmission, but if transmission occurs, rabies follows inevitably. Early symptoms can include fever and tingling at the site of exposure. These symptoms are followed by one or more of the following symptoms: violent movements, uncontrolled excitement, difficulty in drinking water, confusion and loss of consciousness. Once clinical symptoms appear, rabies is virtually 100% fatal within hours or days.

Rabies is present on all continents, except Antarctica. Rabies is one of the Neglected Tropical Diseases that predominantly affects disadvantaged and vulnerable populations who live in rural locations. Approximately 80% of human cases occur in rural areas.

### **RABIES POST-EXPOSURE PROPHYLAXIS**

Rabies transmission is prevented by timely and adequate post-exposure prophylaxis (PEP), which is effective in nearly 100% of cases. Pioneered by Louis Pasteur and his team in the late 1880s, adequate rabies PEP is based on wound cleaning, antisepsis, administration of rabies vaccine, along with rabies immunoglobulin, if indicated.

Rabies PEP, however, all too often remains beyond financial and/or geographical reach, especially for the rural and/or disadvantaged populations of endemic countries, which are most at risk.

An ACIP project funded in 2014 led to the delineation of RESIST (Rabies Elimination Support through Integrative Science and salvage Therapy) research program on rabies PEP, conducted between Institut Pasteur du Cambodge and Institut Pasteur, Paris. Clinical epidemiology and vaccine response studies were carried out and they generated knowledge which provided the grounds for a change in international recommendations for timely and adequate PEP. Since April 2018 the new, double-dose intradermal, vaccine-sparing, one-week/three-sessions "IPC regimen" has replaced the former, already highly effective one month/four sessions "Thai Red Cross" (TRC) regimen.

The remaining question is whether the IPC intradermal (ID) regimen (3 sessions/1-week) confers long-term immunity equivalent to that conferred by a full TRC intradermal regimen (4 sessions/1-month), which was the reference dose-sparing regimen until 2018. This is a concern for public health decision makers and clinical teams in rabies-endemic countries who hesitate to switch to the abridged "IPC regimen".

To date, the rabies virus continues to circulate in 71 countries with a total population of 5.5 B at varying risk of contracting rabies.

Each year, dog-mediated rabies still causes an estimated 60 000 human deaths worldwide, with 95% of cases occurring in Africa and Asia. About 40% of deaths occur in children under the age of 15.

Scientists from instituts Pasteur in Paris, in Cambodia and in Madagascar will associate their expertise to determine in real-world conditions whether the long-term antibody response measured and cellular / humoral immunity parameters are comparable after three intradermal dose-sparing PEP sessions using Vero cell vaccine, compared to that after four sessions, before and after rabies vaccine boosting. To do so, they will identify and recruit patients who received 3 ID PEP versus 4 ID PEP two, five and 10 years earlier in Madagascar and Cambodia. A blood sample will be taken from each patient before and after a single ID boosting session. They will subsequently measure RABV-neutralizing antibody titers in participants before the vaccine booster injection and one week later using two assays (RFFIT as a reference technique and FAVN). They will also investigate the adaptive immune response such as T cell responses and memory B cells before and one week after a rabies vaccine boosting.

Demonstrating the boostability in participants several years after they have received PEP using the IPC regimen compared to the excellent but longer TRC regimen will help health authorities and decision makers adopt this new regimen in endemic countries. Improved access to abridged PEP will reduce costs, improve vaccine equity and spare lives.

An estimated 30 M PEP regimens are administered worldwide each year.

Rural populations in Asia and Africa face daunting obstacles in accessing timely and adequate PEP.



# Identification of therapeutic naturally-occurring compounds and extracts for mitochondrial diseases

Project ACIP nº 443- 2021



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### **PROJECT SUMMARY**



### **KEYWORDS**

Mitochondria, mitochondrial diseases, MD, genetic diseases, drug screening, microalgae, library



### THE MITOCHONDRIAL DISEASES

Mitochondrial Diseases (MD) are multi-systemic genetic disorders that have a population incidence of approximately 1 in 5000, ranking them among the most common inherited metabolic disorders and most prevalent rare diseases. The genetic basis for MD was described more than 30 years ago and today 340 reported disease genes interfere with mitochondrial functions. Mitochondria are responsible for generating over 90% of the energy needed to sustain life and support organ function and metabolically demanding organs. Among those demanding organs, the brain and muscles are often the most profoundly affected tissues in patients with mitochondrial mutations and clinical manifestations can often overlap with more common neurodegenerative diseases including Parkinson's Disease (PD) and Amyotrophic lateral sclerosis (ALS).

### NEED TO FIND EFFECTIVE THERAPEUTIC INTERVENTIONS

There are no cures for MD, and medical interventions are currently aimed at alleviating symptoms. While clinical trials are underway, an urgent need exists to find effective therapeutic interventions. MDs represent the number one form of inherited metabolic disorders, making them one of the most common "rare diseases" and therefore the identification of effective therapies is wide-reaching. Moreover, the underlying molecular and pathophysiological mechanisms of disease that underscore MD demonstrate conspicuous and significant overlap with more common neurodegenerative disorders, including Parkinson's Disease and ALS. Mitochondrial mutations are causal in the pathogenesis of familial forms of PD and ALS. Given these commonalities, therapeutic solutions dedicated to mitochondrial disease may hold similar potential for other neurodegenerative disorders for which curative inventions are also lacking.

### THE PROJECT

The goal of this project is to identify potential therapeutic compounds that can restore the mitochondria's form and function in cellular models of mitochondrial disease (MD). The researchers plan to conduct compound screens directly in human cells using unique collections of compounds and extracts curated by partners in Korea (IPK) and New Caledonia (IPNC). The IPK partnership will provide a library of natural product isolates, including flavonoids, which have been reported to have preventive actions against oxidative stress and mitochondrial dysfunction-associated pathologies. The IPNC partnership will provide access to a collection of 45 pharmacologically active marine microalgae extracts derived from strains collected and established in the New Caledonian lagoon. The project will identify promising therapeutic targets for treating mitochondrial dysfunction in human cell models of disease. More than 300 mitochondrial diseases have been identified.

Mitochondrial diseases can present with a wide range of symptoms and severity, including muscle weakness, seizures, developmental delays, vision and hearing loss, and organ failure...



Assessment of prevalence of Leptospira spp and interaction between environment, animals and human leptospirosis in periurban areas in Africa Project ACIP n° 445- 2021



### SCIENTIFIC COORDINATOR

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### **PROJECT SUMMARY**



### **KEYWORDS**

Leptospires, leptospira, leptospirosis, one health, livestock, epidemiology, environment, rodents, screening, mapping, environment, One Health, sequencing, *Leptospira interrogans* 



### **THE LEPTOSPIROSIS**

Le Leptospirosis is a worldwide bacterial zoonosis caused by Leptospira spirochetes. Humans may contract the disease *via* direct contact with contaminated urine or tissues of infected animals, or indirectly through contaminated soil and water. Leptospira infect animals through skin rashes or mucous membranes. The severity of symptoms varies based on the infection phase, serovar, infectious dose, and host factors. There are 64 phylogenetically defined Leptospira species, categorized into P (pathogenic) and S (saprophytic) clades. P1 subclades are pathogenic to both humans and animals, while P2 induces mild symptoms. Pathogenic leptospires have been detected in over 150 mammalian species, with asymptomatic infection in rodents, cattle, and pigs. Workers in rice fields, irrigated gardens, abattoirs, and veterinarians are at high risk of contracting leptospirosis, which is also prevalent in urban slums with poor sanitary and socioeconomic conditions.

### LEPTOSPIROSIS SURVEILLANCE IN AFRICA

Leptospirosis is a neglected disease in Africa and is likely under-reported in Madagascar and Côte d'Ivoire due to favorable conditions for Leptospira transmission, including climatic, socioeconomic, agricultural activities, and animal husbandry. Rice production is a long-standing tradition in both rural and urban areas of Madagascar and Côte d'Ivoire to ensure local food security. Previous studies have revealed a high prevalence of pathogenic Leptospira in small mammal and livestock populations in both rural and urban areas of these countries. These findings suggest a high risk of leptospirosis transmission to humans. However, human prevalence remains unknown, and risk factors for leptospirosis infection require further investigation.

### THE PROJECT

The project is divided into three aims. First, it will evaluate the prevalence of leptospirosis in periurban areas of Madagascar and Côte d'Ivoire. In a second time, researcher will determine and compare the presence of Leptospira spp in water and soil environments under various environmental and physico-chemical conditions in relation to urban slum contexts. Then, the project will highlight the epidemiology of leptospirosis and identify risk factors associated with human exposure to environmental waters and soils.

This project has the objective to provide valuable insight into the status of Leptospira, transmission and maintenance of the pathogen, and the associated risks to human populations. The findings from this project will help in better controlling the risk of infection and protecting human health.

Approximately one million human cases and 58,900 deaths are reported annually with the prevalence being highest in the tropics, especially during the rainy season.

Leptospires are expected to be one of these bacterial candidates contaminating urban waters.



# Myosins-Usher2 proteins Complexes in hearing Loss Project ACIP n°485-2021



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### **PROJECT SUMMARY**



### **KEYWORDS**

Ears, genetic disease, Usher2 syndrome, cilia, hearing loss, myosins, ADGRV1, PDZD7, whirlin, biophysics, X-ray crystallography, , Nuclear Magnetic Resonance,


### **USHER SYNDROME**

Usher syndrome (USH) is a genetic disorder that affects both hearing and vision and is characterized by congenital hearing loss and progressive visual impairment. USH affects around **1 in 25,000 people worldwide**. There are three types of USH, distinguished by the severity of hearing loss, the presence or absence of balance impairment, and the age of onset of symptoms. **USH2 is the most common type**, accounting for more than 50% of cases in Europe and North America. Individuals with USH2 are born hard of hearing and gradually lose their vision over time. In healthy individuals, cochlear sensory cells in the inner ear have hair bundles composed of regular stereocilia that transform sound vibrations into nerve impulses. In USH2 individuals, these hair bundles are disorganized and tangled, leading to sensory cell dysfunction.

### **USHER PROTEINS GROUP**

The USH2 disease affects the Usher group of proteins that plays a crucial role in the development of hair cells in the ear, which are responsible for hearing and balance. Four specific proteins - WHRN, PDZD7, Usherin, and ADGRV1 - form a complex that contributes to the formation of ankle links that connect the stereocilia, which are essential for proper hearing and balance function. Myosin motor proteins Myo7A and Myo15A help localize these proteins and connect them to the actin cytoskeleton. Usher proteins contain numerous protein-protein interaction domains necessary to the intricacy of the network, but the network's assembly remains elusive, thus leaving the effect of mutations detected in patients to speculation.

### **THE PROJECT**

This project aims to understand the molecular organization of WHRN, PDZD7 proteins and how they interact with Myosins 7A and 15A. The researchers will use structural approaches such as Small-angle X-ray scattering (SAXS), Nuclear Magnetic Resonance (NMR), and X-ray diffraction to study the formation of complexes between these proteins. Additionally, they will investigate pathogenic mutations in the protein sequences, particularly missense mutations, and determine how they affect protein folding, conformation, and interactions. Overall, this research will help to better understand the USH2 disease and may lead to new treatments or therapies for individuals affected by it.

Usher syndrome is responsible of 3-6% of congenital deafness worldwide



Avian arbovirus testing, transmission analysis, and risk mapping Project ACIP n° 489- 2021



### SCIENTIFIC COORDINATOR

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### **PROJECT SUMMARY**



### **KEYWORDS**

Virus, arbovirus, avian, bird, surveillance, diagnostic tool, serology, serosurvey, reservoir, vector, modeling, simulation, biomarkers, mapping



### **ARBOVIRUSES BURDEN**

Arboviruses are a global health concern, causing epidemics in previously unaffected regions. They can cause a range of symptoms, including fever, headaches, muscle and joint pain, rash, and gastrointestinal problems. More severe symptoms, such as polyarthralgia or arthritis, and neurological complications like encephalitis, can also occur. Wildlife, especially birds, can carry arboviruses and act as reservoirs or hosts, leading to infections in mammals, including humans. Several mosquito-borne viruses, such as West Nile, Usutu, Ilheus, Rocio, Saint Louis encephalitis, Oropouche, Mayaro, Tonate, Eastern, Western, and Venezuelan equine encephalitis, are now emerging in South America.

### IMPORTANCE OF SEROLOGIC SURVEILLANCE OF BOTH RESIDENT AND MIGRATORY BIRDS

Avian arboviruses can spread easily from animals to humans, raising concerns about the possibility of these viruses emerging or re-emerging in human populations. Brazil and French Guiana are home to a diverse range of avian species, including migratory birds that follow well-known migration routes. Long-distance spillover of avian viruses has been linked to bird dispersal. In situations where the mosquito-to-bird ratio is low, direct contact between migratory and resident birds is likely responsible for virus transmission. Therefore, monitoring both resident and migratory birds through serological surveillance is crucial to identify potential reservoir species and transmission hot spots.

### THE PROJECT

This project aims to enhance understanding, surveillance, prevention, and control of emerging infectious diseases from avian reservoirs. This project hypothesizes that arboviruses with avian reservoirs will emerge due to local determinants such as habitat modifications and changes in the community structure of reservoir species. Researchers will combine an innovative serological test with virus sampling from avian reservoirs and spatial statistics to further advance the field of zoonotic arboviruses.

The project addresses scientific and technical barriers that have hindered research on avian arboviruses. Specifically, a test will be developed to be adapted to small volumes of bird serum and combine various detection approaches in a single assay, providing an easy, reliable, rapid, and precise detection of viral strains in the avian context.

The project involves screening many avian species from the field. Data collected by serosurveys will help predict the risk of arbovirus transmission among wild birds and identify regions that could be prone to outbreaks.

No human vaccine or treatment exist for these viral illnesses.

This avian diversity of Brazil and French Guiana provides a great opportunity to investigate emergence and spread of avian-borne viral diseases



Association of SARS-CoV-2 variants of concern and COVID-19 severity with the activity of catecholamine biosynthetic/metabolic pathway. Prognostic value and therapeuthic applications.

Project ACIP n° 505-2022



#### SCIENTIFIC COORDINATOR

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### **PROJECT SUMMARY**



### **KEYWORDS**

SARS-CoV-2, COVID-19, virus, catecholamine, L-Dopa decarboxylase (DDC), hypoxia, HIF signaling, oxidative stress, ROS, drug screening, host pathogen interaction, antimicrobial resistance



### THE DISEASE

The coronavirus SARS-CoV-2 causes the COVID-19 disease. Most people with COVID-19 have mild or moderate symptoms and will recover without special treatment. But in some cases, patients have more severe symptoms that will require medical attention. Indeed, symptoms vary from fever, dry cough and dyspnea to acute pneumonia or even neurological manifestations. The virus is identified since late 2019, but the mechanisms underlying virushost interactions remain largely unknown.

### FOCUS ON THE TOPIC

To date, at least 13 vaccines have been developed, but vaccineescaping mutants are emerging. Regarding treatments, five anti-SARS-CoV-2 monoclonal antibodies have been developed and only one of them is effective against the current dominant variant of the virus (Omicron). There is an urgent **need to develop new** therapeutic strategies against SARS-CoV-2. For this purpose, new knowledges of the virus-host interaction are required to develop efficient treatments.

About this, teams of this project have previously demonstrated that COVID-19 severity is inversely related to a key factor of catecholamine biosynthesis in human cells, which is regulated by hypoxia signaling, determinant for immune mechanisms and cell death. Interestingly, catecholamines deregulation implies hypertension, cardiovascular disease, immune dysregulation... However, these symptoms may arise in case of severe COVID-19 disease. These observations allow the teams to propose the analysis of the link between SARS-CoV-2 infection, catecholamine biosynthesis/metabolism and cell's reaction to hypoxia as a potential avenue of exploration for therapeutic purposes.

### **THE PROJECT**

To understand the different degrees of pathogenicity of SARS-CoV-2 strains, the teams will analyze the impact of different strains on catecholamines biosynthesis/metabolism and cell's reaction to hypoxia. The project involves samples from patients with no comorbidities to study the correlation of (1) their viral load and (2) virus-induced symptoms with the above (3) cellular processes. Patients and viruses will be analyzed to identify genetic variations that may explain the pathogenicity of the viruses and the dysregulation of catecholamine biosynthesis/metabolism in patients. A complementary proteomics approach will be performed in a cell model infected with SARS-CoV-2 to further identify mechanisms linking catecholamine biosynthesis/metabolism with viral infection and virus-mediated cell death. In addition, the project will test FDA-approved drugs that target catecholamine metabolism or hypoxia signaling to assess their impact on SARS-CoV-2 replication, thus opening the door to new therapeutic approaches.

COVID-19 counts 383 millions of confirmed cases and 6 million deaths

Catecholamines are notably regulators of blood circulation and pressure.



## Intestinal organoid models for studying host-parasite interaction in Chagas Disease Project ACIP n° 532-2022



### SCIENTIFIC COORDINATOR

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### **PROJECT SUMMARY**



### **KEYWORDS**

Chagas disease, American trypanosomiasis, *Trypanosoma cruzi*, triatomine, chronic-phase, acute phase, parasite, host-parasite, gastrointestinal tract, intestinal epithelium, colon, organoid, mini-gut, cell culture, microscopy, flow cytometry, macrophage, fibroblast, reservoir, drug resistance, pathogen persistence.



### **CHAGAS DISEASE**

Chagas disease (CD), also known as American trypanosomiasis, is a zoonotic and life-threatening tropical illness caused by the parasite *Trypanosoma cruzi*. Human transmission can be congenital, food-borne, made by insect bite, blood transfusion or organ transplant for instance. CD is characterized by a first acute phase with mild, unspecific or absent symptoms and may continue in a chronic phase with serious complications, such as cardiac dysfunction (30% of the cases), digestive and/or neurological alterations (10%). Oral infections are reported as the route leading to the more severe form of the disease. On the other hand, parasites colonizing the colon contribute to persitance and drug resistance. However the physiopathological mechanisms behind these manifestations remain unknown. To date, there are two drugs available against CD acute phase: Benznidazole and Nifurtimox. But there is no specific treatment for the chronic phase.

### FOCUS ON THE TOPIC

The cellular mechanisms driving the colonization of the vertebrates intestine remain poorly understood. So far, in oral transmission and chronic CD, the intestinal tissue appears to have a key role. But the way the parasite persists in the host is still an unsolved question. This situation is partly due to the lack of relevant models for such an analysis. In this context, the organoids can be a game changer. Indeed, the development of intestinal organoids ("mini-guts") offers a potent and relevant, *in vitro* model to study host-parasite interaction within a more physiological context.

### **THE PROJECT**

By using **intestinal organoids** (aka "mini-guts") and derived cell cocultures, the project will provide insights on the involvement of intestinal and organ-related cells on the chronic phase and oral transmission of CD.

*T. cruzi* location preferences will be assessed by infecting organoids from different intestinal regions with fluorescent-reporter *T. cruzi* strains associated with oral outbreaks and with chronic affection of intestinal tissue. Parasite proliferation and cell-specific tropism, as well as the integrity of the mini-guts will be analyzed by microscopy, biochemical and molecular techniques.

The role of immune and stroma cells of the intestinal tissue on *T. cruzi* infection, will be evaluated using co-culture protocols. Mini-guts derived cells will be seeded on a monolayer and co-cultured with macrophages or fibroblasts. Immune inflammatory response states will be assayed by measuring the presence of inflammatory molecules produced by the infected cells in the culture media. Also, the capacity of the parasite to cross these cells' barriers for invading organoid cells will be assessed by performing trans-well infection assays and microscopy observations.

Chagas Disease is endemic in 21 countries of continental Latin America.

Around 7M people are infected by Chagas disease worldwide with a mortality rate over 10,000 deaths per year.



Clinical and parasitological features of cutaneous leishmaniasis with treatment

failure/relapse in endemic foci of Algeria and Tunisia:

focus on oxydative stress markers and immune response

Project ACIP n° 562-2022



### SCIENTIFIC COORDINATOR

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### **PROJECT SUMMARY**



### **KEYWORDS**

Antimony sensitivity, cutaneous leishmaniasis, cytokine, oxidative stress, Nrf2/Keap1, relapsing, parasite, adaptation, treatment resistance, macrophage, *Leishmania major*, HTseq



### THE LEISHMANIASIS DISEASE

Leishmaniasis is caused by protozoa parasites from the *Leishmania* specie. It is transmitted to mammals (humans included) by female sand flies vector, injecting the parasite as they feed on their blood. The macrophages (cells of the immune system) capture the parasite, which continue its development into the cell and will provoke symptoms depending on where the host macrophage location in the body when the parasite is fully develop.

There are 3 forms of the disease: **visceral** (VL), which is the most severe and lead to death in 95% of the cases if left untreated, **mucocutaneous** one leading to a partial or total destruction of mucous membranes of nose, mouth and throat; and the **cutaneous** form (CL), the most common which causes skin lesions, ulcers, life-long scars and serious disabilities and sigma.

### CUTANEOUS LEISHMANIASIS AND DRUG RESISTANCE

**Cutaneous leishmaniasis** can be treated thanks to pentavalent antimony compounds (SbV) if the host immune system is healthy. However, **unnegligeable number of unresponsiveness to the treatment and relapses** cases are a major problem encountered in endemic countries. Nevertheless, mechanisms involved in treatment failures remain poorly known, which risks selecting a drug-resistant parasite strain. Several factors can be involved in the parasite drug resistance, such as an impaired host immune response, host sensitivity to different parasite strains or drugs...

### THE PROJECT

The project aims to understand the **mechanisms involved in** Leishmaniasis antimony treatment resistance, with a focus on the impact of immune oxidative stress response regulation. This will be the first time such an assay will be driven directly from patients sampling in endemic regions (Tunisia and Algeria). Parasite will be isolated directly from skin lesions scratch and patients' blood. Both parasite and patients' blood DNA and RNA will be analyzed before and after treatment to identify parasite adaptations and patients' cells oxidative stress. This project emphases the importance of skills and knowledges sharing through the IPIN thanks to final **cooperative workshop** to be able to link together omics and enzymatic studies results with drug resistance observed on the field. Altogether, this project's results will highlight new knowledges on Leishmaniasis pathological mechanisms, bringing better adapted treatment and patients care. In 2020, 85% of cases covered 10 countries, Algeria and Tunisia included, where the disease is endemic

In the last 5 years 1.2M new cases of CL occurred, with near 1% of relapses



Geographic origin of the parasite *Angiostrongylus cantonensis* and role of the mollusc *Achatina fulica* in its dispersion, in Central and West Africa and in the French departments of America

Project ACIP n° 569-2022



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### **PROJECT SUMMARY**

The use of animal model is applied in accordance with regulatory requirements, the "3 Rs" rule described in Directive 2010/63/EU.



### **KEYWORDS**

Anthropozoonosis, *Angiostrongylus cantonensis*, *Achatica fulica*, parasite, cytB, cytC, oxidase subunit 1, rats, rodents, international, global phylogeny, mitochondrial 16S rRNA

### **INCENTIVE RESEARCH PROGRAMS - SPAIS**



### A. CANTONENSIS AND NERVE ANGIOSTRONGYLOSIS

The nematode Angiostrongylus cantonensis is a parasite of rats (rat lung worm). The adult female parasite lays eggs in rats, which excrete larvae in their feces. Larvae are taken up by intermediate hosts (land snails such as Achatina fulica and slugs) and by paratenic hosts (prawns, frogs, or toads) that can transmit the infection to humans. Human infection is acquired through ingestion of raw or undercooked contaminated intermediate or paratenic hosts. Infection causes Nerve Angiostrongylosis including neurologic manifestations such as eosinophilic meningitis and encephalitis/encephalomyelitis, with fever, vomiting, and ocular invasion. Most of the time, patients have a selflimiting infection and recover completely, but some cases may be fatal, especially in young children.

### THE QUESTION OF A.CANTONENSIS SPREADING

*A. cantonensis* was firstly described in Guangzhou (Canton), China, in 1935 and the first human case of Nerve Angiostrongyliasis has been exposed in Taiwan in 1945. The parasite is endemic in different regions such as Southeast Asia, Pacific Islands, Indian Ocean Islands, the Greater Antilles, Ivory Coast, south states in the USA, French Departments of America (FDA)... Due to a lack of data on the *A. cantonensis* genomic sequences, the history of the dissemination of the parasite through the different continents, and the actual role of potential hosts is still unclear.

### THE PROJECT

The aim of the project is to **determine the origin of the parasite and its way of introduction in French Departments of America and in Africa**. To do so, the project brings together Pasteur Institutes from Africa, Madagascar, Asia-Pacific and FDA. The teams will assess the prevalence of the contaminated hosts in their regions and isolate DNA from host and parasites picked on the field.

Then, the project will build the phylogeny of the parasite strains to elucidate their dissemination history and the role of the host *A. fulica*. The strategy of this project is based on the **skills sharing through workshops** on rats lungs and snail mantle dissection for a good DNA isolation. There will also be workshops on the specific DNA isolation and sequencing of (1) the parasite (exploring cytB and cytC oxydase subunit 1 genes) and (2) its major intermediate host, *A. fulica* (using mitochondrial 16S rRNA gene).

After this project, all the institutes will be able to diagnose *Nerve Angiostrongylosis* which will be helpful to guide clinicians in the management of cases of eosinophilic meningitis. Moreover, the collection of micromammals used for this project could serve subsequently to assess the presence of parasites and especially other species of *Angiostrongylus*, using 18S rRNA gene metabarcoding of rat feces. This strategy would be a way to limit epidemic as it would be robust to detect various parasites' presence in host.

This kind of **cooperative approach is one of the main assets of Institut Pasteur International Network**, bringing together human forces and facilitating scientific knowledge and skills sharing to answer global question. In rare cases, infection by *A*. *cantonensis* leads to a strong inflammatory reaction which causes permanent motor and respiratory systems paralysis

The first case of Angiostrongyliasis in FDA was detected in the very end of 1990's.



## Exploiting Signal-regulatory protein alpha (SIRPA) signaling to control virus-mediated inflammation

Project ACIP n° 570-2022



### SCIENTIFIC COORDINATOR

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### **PROJECT SUMMARY**

The use of animal model is applied in accordance with regulatory requirements, the "3 Rs" rule described in Directive 2010/63/EU.



### **KEYWORDS**

Peptide and Protein synthesis, molecular mechanisms of signaling pathways, innate immunity, cytokine, inflammation, immune signaling virus, host-pathogen interactions, dengue virus, Ebola virus, hemorrhagic fever, SARS-CoV-2, Zika virus, SIRPA, immune response, TLR, macrophage



### **THE DISEASES**

Different RNA viruses, belonging to unrelated viral families, are the causes of human epidemics. Among those viruses, the arenavirus Junín (JUNV), causes the Argentinian Hemorrhagic Fever (fatality rate  $\approx$  30%); Zika virus (ZIKV) and Dengue virus (DENV), flaviviruses are responsible for potentially life-threatening diseases (Zika and Dengue Fevers) infecting millions of people per year; Ebola virus (EBOV), a filovirus causes the Ebola disease, with a fatality rate of 70%; and the coronavirus SARS-CoV-2, is responsible for COVID-19 disease with 400 million cases and a fatality rate  $\approx$  1.5%. Current vaccines and treatments for these diseases, when existing, are challenged by virus variants escaping. In some cases, current treatments may have serious side effects. Even if they are remarkably different, those viruses have a common point: they provoke inflammatory response that may lead to severe cellular and tissue damages.

### FOCUS ON THE TOPIC

Virus-induced **inflammation** involves the production of specific molecules: chemokines and cytokines. They help the infected organism to eliminate the virus. This phenomenon is **highly regulated** and usually does not cause damage to the infected individual. Indeed, mammals acquired robust controlled pathways of their immune response, involving multiple negative regulators. Among them, the **protein SIRPA** (Signal-regulatory protein alpha) is a key actor of the fine-tuned coordination of the immune response that also decreases the viruses' ability to enter human cells. However, the mechanisms driving the activation of SIRPA are unknown.

### **THE PROJECT**

The project aims to better understand how SIRPA acts against viral infections. First, the consortium will investigate how SIRPA is activated by different viral infections in human and mouse immune cell models (macrophages mainly). In a second step, the project will explore the antiviral mechanism of SIRPA. The teams will analyze its impact on the production of key actors of the inflammatory response (cytokines) by macrophages. In addition, using a mouse model, the consortium will investigate how SIRPA protein modulates the inflammatory response upon recognition of viral infection.

Together, the results of this project will provide new informations on the actors and signaling pathways involved in the limitation of viral infections. They will also open new avenues for the development of antiviral therapies, which are essential to limit future viral epidemics.

Fitness-enhancing mutations arised within a few months of the evolution of SARS-CoV-2 within the human population

As an inhibitor of the immune system, SIRPA has also a key role in selfrecognition phenomenon



# Characterization of the Uruguayan population using low-pass sequencing

Project ACIP n° 577-2022



### SCIENTIFIC COORDINATOR

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### **PROJECT SUMMARY**



### **KEYWORDS**

Genetic, genetic of population, sequencing, Latin Americans, admixed population, low pass sequencing, LPS, human trait, ancestry, history, representation, algorithm, population characterization, variant traits



### **BENEFITS OF A ROBUST GENOMIC CHARACTERIZATION**

Since 2001, the **human genome** data is accessible. This crucial scientific breakthrough has led to groundbreaking innovations in the understanding and **treatment of genetic diseases**, and in human history. Initiatives such as *1000 Genomes Project*, compiling human DNA from people around the world, are used as references to identify genetic variations and their consequences. To be as effective as possible and truly **representative of the diversity of humankind**, databases such as 1000 Genomes need to be updated with DNA from as many human populations as possible. This raises the question of the **accessibility of genome sequencing technology to low-income countries**, leading to a selection bias on data collection. Consequences of this situation are the selectivity of the advancement of genomic knowledge, the relevance of which can be undermined by this selection bias.

## DATA BIAS AND CONSEQUENCES: THE CASE OF THE SUB-REPRESENTATION OF URUGUAYAN PEOPLE

When looking at the genomic data produced, a bias is evident. Indeed, **78%** of Genome Wide Association Study (GWAS) concern European individuals, and less than 4% have Latin Americans with indigenous ancestry. This questions the application of those studies on underrepresented populations, such as Latin American people. For instance, there is a clear gap in the application of genomic medicine results because of the lack of knowledge on those people genomes. Another benefit of a better characterization of admixed people's genome would be a new light on ancestries' history. In the specific case of Uruguay, this country led a genocidal politic in the past towards indigenous people, and it was believed that the country was "indigenous-free". The coordinator's project laboratory already demonstrated that a significant part of Uruguayan people present indigenous ancestry.

### **THE PROJECT**

The project proposes to access to a **better characterization of Uruguayan people's genome thanks to the Low Pass Sequencing (LPS)**. Indeed, this method is less expensive and can provide accurate data thanks to an ad hoc imputation algorithm. The project will combine high coverage already sequenced genome references coming from a limited but representative panel to implement the algorithm which will be used. This implementation will limit the potential bias errors on the following analysis of nearly 850 people genomes coming from all the 19 Uruguayan departments.

This project will provide a **panel of reference of currently underrepresented Uruguayan people on genome** analysis which will **reduce the inequalities of global genome analysis applications** Moreover, the project will bring new data on Uruguayan's specific genome signatures, and provide more details on Uruguayan ancestry history. Testing LPS strategy accuracy in this context will allow **new route for a more affordable genome sequencing**, which will benefit to the construction of a more representative model of humankind genome. In 2020, Ben-Eghan *et al.* highlighted that 45 of 58 genome analysis using UK biobank excluded data from specific populations. 31 of them didn't explain this decision.

European genetic prediction accuracy is lower by 1.6-fold lower in in Hispanic/Latino Americans population.

PORTFOLIO TRANSVERSAL RESEARCH PROGRAMS (PTR) FUNDED FROM 2014 TO 2022

### Characterizing the molecular organization of protein networks in hair cells to understand physiopathogenesis of Usher syndrome for developing therapeutic approaches



### Project PTR n° 483-2014

Scientific coordinator: Dr Nicolas WOLFF, Signalling and Molecular Interactions Group - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Barakat ABDELHAMID, Institut Pasteur in Morocco, Dr Amel BAHLOUL, Dr Patrick ENGLAND (Molecular Biophysics Platform) and Dr Jacques BELLALOU (Recombinant Proteins in Prokaryotic Cells Platform), Institut Pasteur (Paris)

### WHAT IS USHER SYNDROME?

Usher syndrome is a rare genetic disorder caused by mutations in genes, involved in the function of the inner ear and retina. It is an autosomal recessive disorder strongly associated with the consaguinity.

Usher syndrome is the most common condition that affects both hearing and vision, sometimes also balance. The disease begins at birth and affects both girls and boys. This syndrome is characterized by hearing loss and a progressive visual impairment that progresses to blindness. The hearing loss is caused by a defective inner ear, whereas the vision loss results from retinitis pigmentosa (RP), a degeneration of the retinal cells. The first symptom of the syndrome is deafness. It can be the only sign of the disease for several years, until the progressive onset of visual disturbances. There are three different forms of Usher syndrome that depend on the age at which the disease evolves, the severity of the hearing loss, and whether there are balance disorders.

The simplest approach to diagnosing Usher syndrome is to test for the characteristic chromosomal mutations. Parental consanguinity is a significant factor in diagnosis. Currently, there is no cure for Usher syndrome.

### HAIR CELLS AND PROTEIN ACTORS IN USHER SYNDROME

The hair cells are the auditory sensory cells which are present in the receptor organ of hearing, called organ of Corti, in the inner ear. They are essential for the transmission of sound to our brain by converting sound-induced vibrations into a nerve signal circulating in the auditive nerve : it is the mechanotransduction. When the hair cells are destroyed, they are not replaced leading to problems of hearing or even deafness.

The study of deafness genes, especially those for Usher syndrome, has helped to identify several structural proteins involved in the development and function of hair cells. One feature of the modular USHER proteins is the capacity to interact with each other through protein-protein interaction domains called PDZ and/or PDZ Binding Motifs (PBM). This protein network in the hair cells is essential for the hearing function in the inner ear.

Thus, understanding the molecular mechanisms of the hair cell protein network is an essential issue of hearing science. However, up to now, only few molecular actors are known and the organization of such submembrane complexes essential for the mechanotransduction is still unknown. Usher syndrome affects one in 30,000 people in France.

Usher syndrome is responsible for the majority of deafblindness.

### **THE PROJECT**

This project will be realized thanks to the association of three teams presenting complementary expertises in genetics (Institut Pasteur in Morocco), structural biophysics and cellular biology (Institut Pasteur Paris) with the support of two Institut Pasteur core facilities. These scientists aim at characterizing the organization of submembrane complexes involved in the mechanoelectrical transduction machinery. For this, they will identify new proteins expressed in the hair cells by genetic approaches, studying the deafness in Moroccan patients. This population is associated with high level of consanguinity that favors autosomal recessive disorders like the USHER syndrome. The consortium will establish the entire human PDZ-PBM interaction network (interactome) in the auditory sensory cells using an efficient and innovative high throughput chromatographic assay that they recently developed. In parallel, the teams will decipher the functional and structural role of two key proteins expressed in the stereocilia (structure of hair cells) and playing an essential role in the mechanoelectrical transduction machinery.

This project should improve knowledge of molecular mechanisms involved in the pathophysiological development and functioning of cochlear sensory hair cells. Characterizing the molecular organization of the Usher complex would have a potential impact on Usher diagnosis, physiopathology and therapeutic approaches. Finally, these findings would provide new clues to understand the physiopathogenesis of Usher inducing hearing loss and might be valuable for developing new specific therapeutically approaches in the future.

## Study the circulation of enteroviruses A71 and assess the epidemic risk in Africa for the implementation of preventive strategies



### Project PTR n° 484-2014

Scientific coordinator: Dr Francis DELPEYROUX, Biology of Enteric Viruses Unit - Institut Pasteur (Paris) Institut Pasteur International Network (IPIN) collaborators : Dr Henda TRIKI, Institut Pasteur in Tunis, Dr Richard NJOUOM, Pasteur Centre in Cameroon, Dr Jean-Michel HERAUD, Institut Pasteur de Madagascar, Dr Leila ANES-BOULAHBAL, Institut Pasteur in Algeria and Dr Edgard Valery ADJOGOUA, Institut Pasteur in Ivory Coast \*

### WHAT IS ENTEROVIRUS A71?

Enterovirus A71 (EV-A71) is an emerging pathogen, member of enterovirus A species in the *Picornaviridae* family. Enteroviruses are divided into seven genogroups (or variants) named A to G. Genogroups B and C contain hundreds of EV-A71 strains isolated worldwide. Genogroup C is currently circulating widely in Asia, and has been implicated in major epidemics. The genogroups E and F have been recently discovered in Africa and Madagascar.

This virus affects mostly children, causing large outbreaks of hand, foot, mouth disease (HFMD), characterized by benign maculopapular lesions. However, in many cases, EV-A71 elicits neurological complications such as encephalitis, potentially leading to severe pulmonary edema, which is often fatal in children. The virus spreads through contact with an infected person's stool and respiratory secretions, such as saliva, nasal mucus or sputum.

Currently, no specific prophylaxis or treatment for EV-A71 is available but promising data have recently been reported in the development of vaccine in China

### **ENTEROVIRUS A71 IN AFRICA**

Although EV-A71 has been reported in many parts of the world, outside the Asian-Pacific region, it has never been shown to be implicated in severe epidemics in African countries.

Collaboratives studies involving teams within the Institut Pasteur International Network (IPIN) have led to the discovery of one EV-A71 isolate of genogroup C in Cameroon, confirming the potential circulation of this genogroup in the African continent. These findings as well as the discovery of genogroups E and F indicate that other EV-A71 genogroups are circulating in Africa and probably in other developing countries with no reported epidemic outbreak.

It is important to determine the extent to which these new EV-A71 genogroups are circulating and whether there are more, as yet unidentified, genogroups circulating within the African populations. Furthermore, analyses comparing the pathogenicity and antigenicity of genogroups E and F with those of genogroups B and C are of great potential interest to evaluate the risk of epidemics due to these new genogroups in Africa.

Worldwide, EV-A71 is a common cause of hand, foot, and mouth disease in infants and young children.

Some people infected with enteroviruses have no symptoms but still can spread the virus to others.

\* This project also involves / involved the participation of external partner(s).

### **THE PROJECT**

The scientists of six institutes within IPIN having a great expertise on enteroviruses propose to study the circulation and genetic diversity of African EV-A71 and to assess the epidemic risk for the population, particularly children exposed to these viruses. For this, a tool adapted to African strains is being developped by one of the consortium partners for detection and diagnostic and then made available to all partners. Thanks to this tool, they will look for the presence of EV-A71 strains in the samples (stools or blood) of children with encephalitis and/or HFMD or samples obtained through poliomyelitis surveillance activities in order to determine which strains cause disease (HFMD and encephalitis). A phylogenic analysis will be carried out on isolates identified previously to study the diversity, the evolution and the circulation of EV-A71 in Africa. Then comparative analyses between classical genogroups (B and C) and new African genogroups (E and F) will be realized to define the genotypic, phenotypic and pathogenic features of the new Africans genogroups. They will also determine the presence of neutralizing EV-A71 antibodies in sera from children and young adults. The sera positive for EV-A71 antibodies will be tested for the capacity of neutralizing antibodies directed against a given genogroup to neutralize homologous isolates or isolates of a different genogroup.

The data obtained from this project will increase knowledge about the diversity and distribution of EV-A71 around the world. This work will make it possible to evaluate the extent to which EV-A71 strains are circulating in Africa, their role in disease and the risk to African children and also implement prevention strategies.

# Understand *Plasmodium vivax* invasion mechanisms to develop a vaccine



### Project PTR n°490-2014

**Coordinator:** Dr Chetan CHITNIS, Malaria Parasite Biology and Vaccines Unit – Institut Pasteur (Paris) **Institut Pasteur International Network collaborators:** Dr Inès VIGAN-WOMAS, Institut Pasteur de Madagascar and Dr Didier MENARD, Institut Pasteur in Cambodia

### **PLASMODIUM VIVAX**

*Plasmodium vivax* (*P. vivax*) is the second agent responsible for malaria, which is present mainly in Asia and Latin America.

*P. vivax* is transmitted to humans by bites of infected female *Anopheles* mosquitoes. This parasite can remain dormant in the liver and activate and invade the blood several months or years after the infecting mosquito bite. *P. vivax* can lead in some cases to severe disease and death.

Unfortunately to date, the fight against this parasite is difficult because no preventive or therapeutic treatment exists that can protect ant treat fully against this disease.

In addition, the control of the vector is hampered by the emergence of mosquito resistance to insecticides.

### VACCINE

Currently, no efficient commercial vaccine is available to ensure the protection of populations in endemic countries and travelers. However, several vaccine candidates based on individual parasite antigens, which are molecules considered as foreign by the body and triggering an anti-parasite immune response, have shown only a limited level of protection. For example, an experimental vaccine against *P. falciparum*, RT, S/AS01, targeting the *sporozoite* form, was developed and has now completed a clinical phase III trial. However, this vaccine does not provide total protection against the parasite. It is therefore necessary to develop a vaccine candidate with higher protective efficiency.

Indeed, *P. falciparum* is not the only problem, there is also *P. vivax*. This parasite was recently discovered in great apes of Central Africa, raising fears of the existence of a natural reservoir with new transfers from these primates to African populations, pointing to a possible risk of emergence. It is necessary to protect the populations from this parasite

### **THE PROJECT**

Teams from Institut Pasteur International Network together with team from Institut Pasteur (Paris) led by Chetan Chitnis will study which key proteins of the parasite are involved in the infection of the reticulocyte (immature red blood cell) at the functional and immunological levels. By better understanding the parasite - host cell interactions, the scientists will study the feasibility to develop a vaccine against the *merozoite* form of the parasite, that will block the penetration of *Plasmodium vivax* in red blood cells. This vaccine will be of a great help to control and eliminate malaria in most endemic countries.

More than one billion people are now exposed to *Plasmodium vivax*.

About 90 million cases of malaria are caused by P. vivax worldwide each year.

## Determine the role of PBMCs in transmission/ infection of dengue vectors for better prevention and control



### Project PTR n° 491-2014

Scientific coordinator: Dr Marc GRANDADAM, Arbovirus and emerging viral diseases unit - Institut Pasteur in Laos Institut Pasteur (Paris) collaborators: Dr Valérie CARO and Dr Anna-Bella FAILLOUX \*

### WHAT IS DENGUE DISEASE?

Dengue is an illness caused by a virus that is spread through female mosquito bites of the *Aedes* type (particularly *A. aegypti* but also *A. albopictus*). Dengue virus is a permanent threat in tropical and subtropical countries worldwide.

The mosquito that carries the dengue virus can bite during the day and night, both indoors and outdoors and often lives around buildings in urban areas. Infected humans are playing the role of virus reservoir in urban and rural areas as long as they support an active multiplication of dengue virus.

There are 4 distinct, but closely related, serotypes of the virus that cause dengue. Recovery from infection by one serotype provides lifelong immunity against that serotype. Subsequent infections by other serotypes increase the risk of developing severe dengue.

Symptoms include fever, headache, nausea, vomiting, rash, and pain in the eyes, joints, and muscles. After the mosquito's bite, symptoms can take up to 2 weeks to develop but usually end in a week. In severe cases, symptoms may include intense stomach pain, repeated vomiting, bleeding from the nose or gums, and death.

Dengue can be diagnosed by direct detection of the virus or by serological tests on serum or plasma samples. However, the sensivity of direct detection methods dramatically decreases from day 5 after fever onset.

There is currently no specific treatment or vaccine available for fighting this disease. The only existing means of fighting the disease are the control of mosquito vectors in the regions concerned and individual measures to protect against mosquito bites.

### **DENGUE VIRUS AND BLOOD CELLS**

The cells considered as the primary cell targets of dengue virus after transmission of mosquito to human are dendritic cells, monocytes and macrophages (immune system cells). Detection of antigen in Peripheral Blood Mononuclear Cells (PBMCs) has been used for the rapid and early diagnosis and as a predictive marker of severity. Some studies have shown that the virus may persist in PBMCs after day 5, but the frequency and the kinetic of these prolonged viremia have not been determined. The importance of peripheral blood cells infection by dengue viruses has been poorly investigated in terms of diagnostic, pathophysiology and maintenance of the viral cycle. As a consequence, the role of PBMCs in the mechanism of mosquito infection is unkown.

Two and a half billion people live in risk areas. Although initially observed in the tropical and sub-tropical regions of the world, dengue has now reached Europe.

Dengue fever is mostly benign, but severe and unpredictable forms occur in about 1% of cases, more often in young children.

\* This project also involves / involved the participation of external partner(s).

### **THE PROJECT**

The scientists from IP Laos and IP Paris will join its complementary competence to study a particular blood population (PBMC) in patients during dengue infection to determine the role of these blood cells in infection process of naive mosquitoes. Firstly, the scientists will establish the kinetic of dengue virus infection patients' PBMCs. These patients recruited within clinical facilities will be followed from the early phase to the convalescent phase by quantifying and isolating the virus. They will then study the evolution of the genetic diversity of viruses in human samples as well as in mosquito samples using a high throughput sequencing approach. At last, they will explore the ability of infected PBMCs to transmit dengue virus to mosquitoes.

This project will provide new knowledge regarding the mechanism of the virus transmission and dengue pathophysiology in humans that may have an impact for the diagnosis and the prevention of dengue transmission.

### How gastric *Helicobacter* species have evolved to be the sole colonizer of a unique niche, the stomach in half of the human population



### Project PTR n° 494-2014

Scientific coordinator: Dr Daniel VINELLA, *Helicobacter* Pathogenesis Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Julia CHAMOT-ROOKE (UTechS Mass Spectrometry for Biology), Institut Pasteur (Paris), Dr Frédéric VEYRIER, INRS – Institut Armand Frappier (Canada)

### WHAT IS HELICOBACTER?

*Helicobacter* is a genus of Gram-negative bacteria belonging to the Epsilonproteobacteria class. This genus encompasses species having either a gastrointestinal tract and/or liver tropism that colonize mammals and birds or a gastric tropism in several mammals.

The most studied species of the genus is the gastric species *Helicobacter pylori* (*Hp*) that infects the stomach of humans causing chronic gastritis, peptic ulcer disease, MALT lymphoma and stomach cancer.

*Hp* is transmitted from person to person by direct contact. *Helicobacter pylori* infection usually occurs in childhood. The symptoms may include bloating, nausea or vomiting, stomach pain, fatigue and a feeling of fullness after eating a small amount of food. This bacteria is diagnosed by blood, breath and stool tests. The treatment involves the combination of two antibiotics associated to medications to decrease the amount of the acidity in the stomach.

#### SURVIVAL IN GASTRIC ENVIRONMENT

All gastric *Helicobacter* species possess two enzymes, urease and [NiFe]hydrogenase, that have been shown to be essential for colonization by *Hp*. Urease neutralizes the acidic pH encountered in the stomach. These two enzymes require nickel (Ni) as a co-factor for their activity and thus for the survival within the stomach of *Hp*, and likely of other gastric *Helicobacter* species. Therefore, the gastric *Helicobacter* species depend on an important supply in Ni. *Hp* possesses original mechanisms for Ni scavenging, uptake and storage as well as for delivery and incorporation into these two enzymes that are partially understood but some actors have already been identified. For Ni transport across its outer-membrane, *Hp* requires a transporter which is acid-induced allowing to optimize urease activity by Ni incorporation under conditions where urease activity needs to be maximal. *Hp* possesses two small proteins, extremely rich in Histidine (His) residues having strong affinity for Ni. These two proteins have different functions, one participates in the storage of Ni and in urease activation and the other acts in regulation of nickel trafficking.

Coordinator team has been studying for several years the mechanisms of Ni metabolism in *Hp*. Recently, they published that evolutionary separation of the gastric *Helicobacter* species from the other *Helicobacter* species occurred concomitantly with the acquisition of small His-rich proteins and of nickel transporter. These observations prompted they to ask new and original questions on the evolution of the *Helicobacter* species and their hosts : are these three events related? do they result from an adaptation of the bacteria to their hosts? If so, how did this occur?

*Hp* infects the stomach of up to 50% of the human population worldwide.

Persistent colonization by Hp is associated with the development of various gastric pathologies causing about one million deaths per year in the world. Therefore, eradication of *Hp* has become a public health concern.

### **THE PROJECT**

This project brings together two team from IP Paris and one from INRS (Canada) with complementary expertise (bioinformatics, genetics, biochemistry and mass spectrometry) to identify what differentiates *Hp* and gastric *Helicobacter* bacteria from the non-gastric *Helicobacter* focusing on understanding changes in nickel-related pathways. Firstly, they will characterize the genomic differences between gastric and non-gastric *Helicobacter* species to identify the acquisition or loss of events that accompanied the *Helicobacter* gastric adaptation. They will establish the repertoire of Ni-binding and His-rich proteins in gastric and non-gastric *Helicobacter* species to provide clues on the mechanisms of their emergence and on their roles. Then they will test the *in vivo* role of some of these His-rich proteins and will start to determine the interaction network of the Ni-binding proteins in *Hp*.

This project will generate novel scientific knowledge that will be beneficial for the large community studying metalloproteins and metal trafficking in bacterial, archaeal and eukaryotic organisms. But also, these novel fundamental knowledge might potentially be relevant for the study of metal uptake and trafficking in multicellular eukaryotes such as humans in which several diseases (Alzheimer) have been associated with metal dyshomeostasis.

## Understand the interaction between *Leishmania* and its host cell to identify new therapeutic pathways



### Project PTR n° 496-2014

**Coordinator:** Dr Éric PRINA – Molecular Parasitology and Signaling Unit - Institut Pasteur (Paris) **Institut Pasteur International Network collaborators:** Dr Guangxung MENG, Institut Pasteur of Shanghai-Chinese Academy Sciences, Dr Nathalie AULNER (UTechS Photonic BioImaging) and Dr Laurence FIETTE, Institut Pasteur (Paris)

### WHAT IS LEISHMANIA AND LEISHMANIASIS?

Leishmaniases are chronic diseases caused by *Leishmania* parasites, that are transmitted to different vertebrates, including humans and various animals, by the bites of infected female phlebotomine sandflies. These parasites are prevalent in all parts of the globe, and in Europe they are found around the Mediterranean Basin.

Leishmaniases appear in different clinical forms that are classified mainly in two categories. Cutaneous leishmaniasis is the most common form and causes skin lesions, mainly ulcers, on exposed parts of the body, leaving life-long scars and serious disability when affecting mucosal tissues. Visceral leishmaniasis is the most severe and fatal form in the absence of treatment and is characterized by fever, enlargement of liver and spleen, and anemia.

The incidence of leishmaniasis is increasing worldwide. This alarming situation is the result of spread of the insect vector due to climate change and the displacement of infected populations from disease endemic to non-endemic areas. In addition coinfection with HIV intensifies the burden of visceral and cutaneous leishmaniasis by causing severe forms that are more difficult to manage.

To date, few solutions exist to treat any form of the disease but the treatments currently available are highly toxic and inefficient due to the emergence of drug resistance parasites. Furthermore, no vaccine and no preventive treatment exist to protect the 350 million people exposed or at risk of contracting this disease.

## RELATION BETWEEN *LEISHMANIA* PARASITES AND THE IMMUNE SYSTEM OF THE HOST

After a bite by infected sandflies, *Leishmania* enters and develops mostly in macrophages (primary host cells) that are manipulated by the parasites to become permissive to parasite development and growth.

Macrophages are key players in the immune responses to pathogens and their activation state plays a key role in the outcome of leishmaniasis. The subversion and evasion of its defense mechanisms are essential mechanisms for parasite survival and pathogenesis. Additionally, *Leishmania* parasites have evolved various strategies to hijacking some of the host cell's essential cellular functions for their own benefit. Understanding these interactions and deciphering innate and immune evasion strategies may open new potential therapeutic pathways.

More than 12 million humans are currently infected in the world.

*Leishmania* parasite causes 1,3 million new cases and the death of 40,000 people per year.

Populations at risk of visceral leishmaniasis are children from 1 to 3 years



### **THE PROJECT**

Teams from the Institut Pasteur International Network (Institut Pasteur Paris and Institut Pasteur Shanghai) will combine their different expertise to decipher the subversion strategies used by *Leishmania*, including inhibition of the macrophage's anti-microbial activities or interference with the induction of an antileishmanial immune response. Scientists will study the regulatory mechanisms involved in *Leishmania* survival in macrophages. More particularly, they will focus on the mechanisms connected with the inflammasome, a multiprotein complex that is a key player in innate immune system.

This new knowledge will allow the discovery of new avenues to stimulate and rescue antimicrobial functions of infected macrophages to eliminate intracellular *Leishmania* parasites.

A better understanding of the parasite and host parasite interactions will have an impact in the development of new drugs.

## Understand host-hantavirus relationships and pathogenesis for humans and better monitor these viruses



### Project PTR n° 505-2014

Scientific coordinator: Dr Noël TORDO, Unit of Antiviral Strategies - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Jean-Michel HERAUD, Institut Pasteur de Madagascar, and Stéphane PETRES (Production and Purification of Recombinant Proteins Technological Platform), Institut Pasteur (Paris)

### WHAT ARE HANTAVIRUSES?

Hantaviruses are emerging zoonotic pathogens belonging to *Hantaviridae* family, *Bunyavirales* order distributed worldwide except in Antartica. There are today 41 recognized hantavirus species that differ in their geographic distribution and their virulence, natural hosts and several are known to be pathogenic for humans.

The natural hosts of Hantaviruses are rodents (rats, mice, voles,...), insectivores (shrews, moles...) or bats that have a non pathogenic chronic infection. They are excellent reservoirs en vectors of the virus they excrete in urine, feces or saliva. Human contamination is usually caused by inhaling the virus present in the excreta of rodents, during activities in the forest or in premises long uninhabited near the forest.

The symptoms start with mild signs like flu (fever, headache, aches, abdominal pain, diarrhea, vomitings,...) 1 to 8 weeks after virus exposure. In some cases, the infection progresses to severe diseases such as Haemorrhagic Fever with Renal Syndrome (HFRS) and Hantavirus CardioPulmonary Syndrome (HCPS), respectively causing renal failure or pulmonary edema, which can be fatal.

Currently, there is no specific treatment nor vaccine preventing hantavirus infection.

### HANTAVIRUSES-HOSTS RELATIONSHIPS

Europe hosts several pathogenic hantiviruses that cause HFRS of different severity (e.g. Dobrava virus - DOBV), as well as other presumably apathogenic for humans (Tula virus - TULV). DOBV is linked to very severe cases of HFRS in the Balkans and South-Eastern Europe. The presence of its natural host in Western and Central Europe suggests that DOBV may be either already present in these regions, or ready to spread under favorables conditions. Therefore, it is extremely important to understand the mechanisms responsible for the transmission of DOBV between rodents and also the spill-over from rodents to humans.

Furthermore, the host range of hantaviruses is much wider than previoulsy thought, not limited to rodents, but shrews, moles and bats were found to host highly divergent genotypes. It is therefore extremely important to understand the hantavirus geographic distribution and association with different host species and human diseases to evaluate the public health risks linked to hantavirus emergence. While geographic distribution and epidemiology of hantaviruses in Europe and Americas are relatively well investigated, only few studies have focused on Africa and South-East Asia. Large-scale serological studies would be extremely useful in Madagascar that represents a unique biotope.

Finally, for many years, the progress in understanding hantaviruses biology and host association has been hampered by the lack of genetic tool.

Four principal species of hantaviruses circulate in rodents on the European continent, Puumala virus (PUUV), Seoul virus (SEOV), Dobrava virus (DOBV) and Tula virus (TULV). Three of them have been detected in France.

In Europe, most of the 10000 annual HFRS cases are caused by PUUV but DOBV is the second major hantavirus and is highly pathogenic in human.

Depending on the virus, the death rate ranges from <1% to >12%.

An estimated 150,000 to 200,000 cases of HFRS occur each year worldwide.

### **THE PROJECT**

The scientists from Institut Pasteur (Paris) and Madagascar will combine their expertise (virology, production and purification of proteins, field sampling and epidemiology) to understand host-hantavirus relationships and their pathogenicity for humans. It is planed to develop reverse genetics systems (minireplicon (MRS) and rescue (RS) systems) for two European hantaviruses (the highly pathogenic DOBV and the apathogenic TULV), carried by two distincts rodent hosts, respectively. The MRS system will be used to identify viral sequences important for transcription, replication as well as viral protein snythesis. The RS system will be employed to study the role of the different proteins in the hantaviruses (DOBV, TULV...) in order to develop serological tools to estimate the prevalence of hantaviruses in human population in Madagascar as well as their circulation among rodents, insectivores and bats.

This project will provide tools for i) studying the host specifity of hantavirus and pathogenesis for humans and ii) screening human samples for evidence of hantaviral infections. It will also generate important knowledge concerning distribution, evolution and epidemiology of hantaviruses in Madagascar as a model location in the Indian Ocean.

# Biomarkers for Detection Test of Pathogenic *Bacillus cereus* Strains



### Project PTR n° 510-2014

**Coordinators:** Dr Dominique CLERMONT–Biological Resource Center of the Institut Pasteur (CRBIP)– Institut Pasteur (Paris) and Dr Sabine HERBIN, Laboratory for Food Safety – ANSES (French Agency for Food, Environmental and Occupational Health and Safety)

### **THE CONTEXT**

The *Bacillus cereus sensu lato* group includes several genetically closely related species, and contains a spectrum of strains ranging from non-toxicological to hazardous strains. *Bacillus cereus* is the second most frequently suspected pathogen in food-poisoning incidents. Currently, there is no diagnostic test to differentiate innocuous from highly toxic *Bacillus cereus* strains.

### **THE PROJECT**

The project aims at the delivering of new tools to discriminate high pathogenic from non-toxicological *Bacillus cereus* strains. The study fills a crucial need to improve characterization of food poisoning outbreaks caused by *Bacillus cereus*.

The data analyses, obtained from proteomics, Next-Generation Sequencing (NGS) and microbiology studies, will provide biomarkers linked with high pathogenicity and species differentiation within the *Bacillus cereus* group. This study is based on a collection of 100 *Bacillus cereus* strains issued from various locations (food, clinics and environment). A database referencing *Bacillus cereus* strains will be created following their proteomics (MALDI-TOF MS analyses) and toxicological profiles (toxicological cell-based assays).

Hazardous *Bacillus cereus* strains may cause food-poisoning incidents, from emetic or diarrheal-type of food-borne illnesses to more serious and may even lead to cases with lethal complications, especially for the most vulnerable populations such as that of children. Biomarkers identified will be used for the development of specific tests to detect pathogenic *Bacillus cereus* strains in food samples at any stage of their manufacture. For the first time, virulence characteristics of *Bacillus cereus* strains could be predicted.

Identification of biomarkers to be targeted in link with the pathogenicity and species differentiation within the *Bacillus cereus* group.

#### **PUBLICATIONS:**

- Cadel Six et al., A food poisoning outbreak investigation due to enterotoxin producing Bacillus cereus, Bulletin épidémiologique, 2014
- Cadel Six et al., Toxi-infections alimentaires collectives à Bacillus cereus : bilan de la caractérisation des souches de 2006 à 2010, Hors série BEH, 2012
- Clermont D. et al., Multilocus sequence analysis of the genus Citrobacter and description of Citrobacter pasteurii sp. nov., Int J Syst Evol Microbiol., 2015 May;65(Pt 5):1486-90.

## Identification of novel mechanisms controlling bacterial infection by exploring the *Listeria* model system



### Project PTR n° 521-2015

**Coordinator:** Dr Javier PIZARRO-CERDA, Cell-Bacteria Interactions Unit - Institut Pasteur (Paris) **Institut Pasteur International Network collaborators:** Dr Hugo NAYA, Institut Pasteur in Montevideo, and Dr Spencer SHORTE (UTechS Photonic Biolmaging), Institut Pasteur (Paris)\*

### WHAT IS LISTERIOSIS?

Listeriosis is a serious infection usually caused by eating food contaminated with the bacterium *Listeria monocytogenes*. Although killed by heating, the bacterium is able to multiply at 4°C. The foods most often contaminated by *Listeria* are delicatessen meats (tongues, rillettes), processed fish products, chilled sprouted seeds and fresh dairy products (soft cheeses and cheeses made with raw milk). This bacterium is present in water, soil and plants.

This disease primarily affects older adults, pregnant women, newborns and adults with weakened immune systems. *Listeria* causes meningoencephalitis and/ or septicaemia in adults and newborns. In pregnant women, it causes fever and abortion. Transmission is also possible from mother to foetus or from a mother to the newborn during birth.

An antibiotic treatment is available, and is more effective when administered early. However, even if targeted with early treatment the outcome may not be as desired and can be fatal.

### **MECHANISMS REQUIRED FOR CELLULAR INFECTION BY BACTERIAL PATHOGENS**

In the early 1990s, small non-coding RNA molecules called microRNAs (or miRNAs) were discovered in eukaryotic cells, meaning cells having a nucleus such as plants and animals. These microRNAs play a role in many biological processes including cell differentiation, metabolism, development and also in infections by diverse pathogens such as viruses, parasites and bacteria. Deregulated microRNA expression is associated with several types of diseases including cancers, immune and metabolic disorders. The microRNA response to bacterial infection diseases has been investigated only in very recent times and the general knowledge in the field is still fragmentary.

### **THE PROJECT**

The teams of Institut Pasteur International Network, including the one led by Javier Pizarro-Cerda, will combine their expertise in the domain of *Listeria* infection, microscopy and bioinformatics to study the role of microRNAs during the cellular infection by *Listeria*. More particularly, the scientists propose the use of the bacterial pathogen model *Listeria monocytogenes* to identify novel gene transcriptional networks involved in the regulation of the bacterial infection in human cells. This project will also implement novel image-based approaches to monitor for example bacterial entry and proliferation in host cells. This novel knowledge will lead to the identification of biologically-relevant cellular targets to control bacterial infection, which is essential for understanding microRNA function and for exploiting their undoubted therapeutic potential.

\* This project also involves / involved the participation of external partner(s).

*Listeria* is responsible for the deadliest bacterial foodborne outbreak in the US (California, 1985).

In 2010, *Listeria* infected 23,150 persons worldwide and killed 5,463 (highest mortality rate among foodborne pathogens).

Biologists use *Listeria* as a molecular model to manipulate cells & to study human biology.

### Understand neurodegenerative processes in humans is a major challenge for science and the society (Example: Parkinson's disease)



### Project PTR n° 523-2015

**Coordinator:** Dr Rebecca MATSAS, Laboratory of Cellular and Molecular Neurobiology - Hellenic Pasteur Institute (Athens, Greece) Institut Pasteur (Paris) collaborators: Dr Chiara ZURZOLO and Dr Pierre-Marie LLEDO

### WHAT IS NEURODEGENERATIVE DISEASE?

Neurodegenerative disease is an umbrella term for a range of conditions which primarily affect the neurons in the human brain. Neurons are the building blocks of the nervous system which include the brain and spinal cord. In neurodegenerative diseases, there is a progressive loss of structure or function of neurons, including death of neurons, which are not replaced. Examples of neurodegenerative diseases include Parkinson's, Alzheimer's and Huntington's diseases. These three diseases manifest with different clinical features, but many similarities appear at the cellular level. There are currently no therapy available to cure neurodegeneration. Medications can only alleviate symptoms and help to improve the patients' quality of life.

### INVOLVEMENT OF THE PROTEIN ALPHA-SYNUCLEIN IN PARKINSON'S DISEASE

Parkinson's disease is the second most common neurodegenerative disorder and manifests as slow movement, rigidity and resting tremor. Non-motor symptoms include smell deterioration, dementia, psychological or psychiatric problems. This disease is a degenerative disorder of the central nervous system. It results from the death of cells generating dopamine (molecule that is essential for movement control) in the substantia nigra, a region located in the midbrain, but also propagates in upper brain regions. The cause of the cell-death is unknown.

Alpha-synuclein ( $\alpha$ -syn) is a protein necessary for communication between nerve cells and plays a central role in certain pathologies such as Parkinson's disease. Neuronal accumulation of  $\alpha$ -syn and formation of intracytoplasmic inclusions termed Lewy bodies are key features in these pathologies. According to a recent hypothesis, the progression of these diseases in the brain could be linked to the propagation of « pathological »  $\alpha$ -syn in Lewy bodies between neurons, similar to what occurs in prion diseases. The transmission mechanisms of  $\alpha$ -syn are poorly studied. Understanding the mechanisms involved in neurodegenerative diseases are very important for science and the society considering the increase of life expectancy and the growth of the aging population worldwide.

### **THE PROJECT**

The consortium, comprising two teams from Institut Pasteur (Paris) and one from the Institut Pasteur in Athens, proposes to combine their complementary expertise to address the question of «naïve» and mutated pathological  $\alpha$ -syn transmission in human neurons. Working together, the three groups will be in a unique position to characterize the mechanism of  $\alpha$ -syn transmission and spreading and to unequivocally link this event to disease progression.

The project will advance the scientific knowledge on Parkinson's disease and will contribute towards the identification of new valuable "druggable" targets to limit disease propagation.

Alzheimer's and related diseases affect over 900,000 people in France and over 35 million in the world in 2010.

In the world, about 5 million people suffer from Parkinson's disease and 300,000 individuals are diagnosed each year.

In France Parkinson's disease affects 1% of the population over 65 years, with 8,000 diagnosed each year.

### Risk of Emergence of Urban Yellow Fever in Brazil: Leading role of the Asian tiger mosquito

## PTR PASTEUR

### Project PTR n° 528-2015

**Coordinator:** Dr Ricardo LOURENCO DE OLIVEIRA, Laboratorio de Transmissores de Hematozoarios - FIOCRUZ (Brazil) **Institut Pasteur International Network collaborators:** Dr Anna-Bella FAILLOUX, Institut Pasteur (Paris), Dr Séverine MATHEUS and Dr Vincent LACOSTE, Institut Pasteur in French Guiana \*

### WHAT IS YELLOW FEVER?

Yellow fever is an acute viral haemorrhagic disease, caused by an arbovirus of flavivirus genus, occurs in tropical regions of Africa and South America and especially in the Amazon basin. The virus is transmitted to humans by bites of infected mosquitoes (genus *Aedes*), during these passages in forest areas (forest cycle, sporadic cases) or directly in cities or villages (urban cycle or suburban source massive and deadly epidemics).

Despite, the availability of effective vaccines, yellow fever remains a major public health problem in Africa and South America since no specific treatment exists to cure the disease.

### **YELLOW FEVER IN BRAZIL**

At first through vaccination and then through an elimination program of the mosquito vector of this disease, urban Yellow Fever was eradicated in 1957 in Brazil and only jungle Yellow Fever persisted. However, in the last 20 years, outbreaks and isolated cases of Yellow Fever have been diagnosed more frequently in areas where it was rare until now. Indeed, the invasion of the mosquito *Aedes albopictus* also called «tiger mosquito» has been observed in regions where human cases have been recently reported, in particular the Atlantic coast.

### **THE PROJECT**

The teams of the Institut Pasteur at the origin of this project, specially the team led by Anna-Bella Failloux who has expertise in genetic diversity of the virus in the mosquito, is going to collect data directly on the field and to shall also lead in parallel experiments in laboratory. This study will also enhance the knowledge of the vector by identifying the species of mosquitoes able to transmit the different virus strains circulating on the field. Thus, the scientists shall be able to determine the risk of emergence of urban Yellow Fever in the region of South America, in particular in regions infested by the Asian tiger mosquito. Ultimately, this study will help in defining more adapted control strategies through prevention and vaccination.

900 million people in the world are exposed to the risk of contracting the virus.

Every year in the world, 200,000 cases of yellow fever among which 30,000 are mortal.

The spread of yellow fever is directly related to the deforestation and to the consequences of the climate change.

\* This project also involves / involved the participation of external partner(s).

## Characterization of *Aspergillus fumigatus* proteins should enable the selection of potential candidates for biotechnological applications



### Project PTR n° 529-2015

**Coordinator:** Dr Anne BEAUVAIS, *Aspergillus* Unit - Institut Pasteur (Paris) **Institut Pasteur International Network collaborators:** Dr Inaki GUIJARRO Institut Pasteur (Paris) and Dr Frank LAFONT Institut Pasteur in Lille (France)

### WHAT IS ASPERGILLUS FUMIGATUS?

Aspergillus fumigatus is the most ubiquitous of the airborne saprophytic fungi able to feed on non-living organic matter. *A. fumigatus* produces small conidia or spores that are very volatile. The conidia are robust and can survive in a wide range of environmental conditions. *A. fumigatus* causes infection in most cases by the inhalation of these conidia present in the environment by human population. They can cause a wide range of diseases, from common allergies to fatal invasive infections. Immunocompromised individuals are especially susceptible to *Aspergillus* colonization that culminates in fatal invasive aspergillosis (IA). IA results in a mortality rate of around 50% even with antifungal therapy. There are currently four classes of antifungal drugs having a relative efficacy against *Aspergillus* species. Reports have shown more and more cases of antifungal drug resistance.

### **HYDROPHOBINS**

Hydrophobins are unique to the fungal kingdom. In *A. fumigatus*, there are seven hydrophobins (RodA-RodG). The role of one of the hydrophobins, RodA, which forms a rodlet layer at the surface of the conidia providing hydrophobicity and masking immunological recognition, has been established unambiguously. The role played by other hydrophobins (RodB-RodG) remains to be investigated.

### **THE PROJECT**

The three teams involved in this PTR proposal will combine their expertise to understand the importance of different hydrophobins in *A. fumigatus*. The project will study the structures, mechanism of self-assembly into rodlets and the functional roles played by hydrophobins B to G in *A. fumigatus* biology and pathobiology. For these studies, they will use special structural biology and biophysical techniques coupled with functional studies. Further, characterization of *A. fumigatus* hydrophobins should enable the selection of potential candidates for biotechnological applications. For example, since RodA renders the spores inert relative to human immune system and resists degradation, this hydrophobin could be an ideal candidate to produce rodletbased nanoparticles for the delivery of immunogenic therapeutic proteins. In this sense, the scientists will use small beads of a highly immunogenic protein from *A. fumigatus*, covered with RodA to try to establish a proof of concept.

Aspergillus fumigatus are involved in 80% to 90% of human Aspergillosis.

Aspergillus fumigatus is responsible for 200,000 deaths per year worldwide.

The mortality rates of invasive Aspergillosis range from 40%-90% in risk groups.

## Decipher *Plasmodium falciparum* mechanism of resistance to Artemisinin



### Project PTR n° 535-2015

**Coordinator:** Dr Jean-Christophe BARALE, Structural Microbiology Unit - Institut Pasteur (Paris) **Institut Pasteur International Network collaborators:** Dr Inès VIGAN-WOMAS, Institut Pasteur de Madagascar and Dr Didier MENARD, Institut Pasteur in Cambodia, Dr Pierre LAFAYE, Antibody Engineering Core Facility, Jacques BELLALOU and Stéphane PETRES Production of Recombinant Proteins Core Facility, Mariette MATONDO-BOUZANDA, Proteomic Core Facility and Ahmed HAOUZ, Crystallography Core Facility, Institut Pasteur (Paris)

### PLASMODIUM FALCIPARUM

Malaria is a fatal parasitic disease is caused by several species of parasites of the genus *Plasmodium* of which *Plasmodium falciparum* and *Plasmodium vivax*.

*P. falciparum* is transmitted to humans by bites of infected female *Anopheles* mosquitoes. After the bite, the parasite infects liver cells where it multiplies. These new parasites are then released into the blood, infect red blood cells and multiply to be released again by bursting of red blood cells, following a continuous infectious cycle. The *Plasmodium* life cycle is very complex with several forms: *sporozoite*, the infectious form injected by mosquitoes and the *merozoite*, the form that infects red blood cells.

The symptoms of malaria, including fever and cerebral malaria, are related to the blood stage of infection by the parasite.

At present, several antimalarial drugs can be used (1) for prophylaxis but do not guarantee absolute protection against infection or (2) as therapeutics decreasing only the duration and severity of the disease. One of the obstacles in the fight against malaria is the resistance of the parasite to current drugs.

### **RESISTANCE TO TREATMENTS**

At the end of 2013, most countries, representing 79 of the 88 countries where *Plasmodium falciparum* is endemic, had adopted the Artemisinin based Combination Therapy (ACT) as first-line treatment. Existing treatments consist of combination therapies that include classic antimalarials (chloroquine, pyrimethamine) combined with artemisinin. The resistance of the malaria parasite *Plasmodium falciparum* to existing treatments is a major global public health problem. Indeed, resistance to classical antimalarials has developed over the last several decades throughout the world: a decrease in the efficiency of ACT was observed in South-East Asia, due to the emergence of *Plasmodium falciparum* resistance to artemisinin.

Recently, one protein involved in *P. falciparum* resistance has been identified but the properties of this protein remain to be investigated.

### **THE PROJECT**

Teams of the Institut Pasteur International Network, led by Jean-Christophe Barale, have a recognized expertise in the field of *Plasmodium*. In previous studies, they identified the protein involved in *Plasmodium falciparum* resistance to artemisinin. During this project, the scientists will develop tools to characterize the biological function and the properties of this protein, particularly in the context of the resistance to artemisinin. The tools currently developed in this project could have an impact in the field to allow a specific diagnostic of artemisinin-resistant parasites, thus allowing to provide the best treatment to patients, but also to follow the dissemination of resistant *P. falciparum*.

Resistance to ACT represents a major threat to public health.

Resistance to ACT will impact the global strategy to fight this disease.

In 2013, 1.5 million cases of malaria in South-East Asia
# Understand the interaction between *Leishmania* and the host cell to identify new drugs



#### Project PTR n° 539-2015

**Coordinator:** Dr Joo Hwan NO, Leishmania Research Laboratory - Institut Pasteur Korea **Institut Pasteur International Network collaborators:** Dr Gérald SPAETH, Institut Pasteur (Paris), and Dr David SHUM, Institut Pasteur Korea

#### WHAT IS LEISHMANIA AND LEISHMANIASIS?

Leishmaniasis is a chronic disease caused by a parasite called *Leishmania* and transmitted to vertebrates, including humans and various animals - mostly dogs - by the bites of infected female phlebotomine sand flies. It is prevalent in all parts of the globe, and in Europe it is found around the Mediterranean Basin.

Leishmaniasis appears in different clinical forms which are classified mainly in two categories: cutaneous leishmaniasis and visceral leishmaniasis (the most severe and fatal form in the absence of treatment). To date, few solutions exist to treat any form of the disease, with the few existing treatments showing important toxicity for patient and rendered inefficient by resistance (the parasite becomes insensitive to the drug). Furthermore, no vaccine and no preventive treatment exist to protect the 350 million people exposed or at risk of contracting this disease.

#### HOW DOES LEISHMANIA PARASITES INFECT THE BODY?

Once inoculated into the body after a bite by infected sand flies, the parasite is taken up by an immune cell well known for its role in the protection against the pathogenic attacks: the macrophage. After phagocytic uptake into the cell, the parasite releases proteins, including ecto-kinases, which interact with factors of the macrophage and deteriorate the immune response of the host. Macrophages thus are exploited by the parasite as host cells for survival and proliferation, which occurs in various organs (skin, liver, spleen, bone marrow).

#### **THE PROJECT**

The teams from Institut Pasteur International Network, including the teams led by Gérald Spaeth and Joo Hwan No, will combine their different expertise to elucidate the mechanisms of the molecular cross-talk between the parasite and the host cells. Scientists will focus on ecto-kinases (signaling proteins released by the parasite) and in particular on their functions in subverting host cell immune pathways. This new knowledge will allow the identification of host substrates of these parasite ecto-kinases and host factors that are involved in allowing intracellular parasite survival, and to evaluate the potential of these factors as novel anti-leishmanial drug targets. A better understanding of the parasite and these interactions with the host cell will have an impact in the development of new drugs. Every year in the world, there were 1,3 million new cases of Leishmaniasis .

Each year 500,000 patients contract the most serious form of the disease.

*Leishmania* parasite causes the death of 20,000 to 30,000 people per year in the world.

## The microbiota: the forgotten organ!



#### Project PTR n° 540-2015

**Coordinator:** Dr Jean-Marc GHIGO, Unit of Genetics of biofilms - Institut Pasteur (Paris) **Institut Pasteur International Network collaborator:** Dr Daniela DE BIASE, Institut Pasteur - Cenci Bolognetti Foundation (Rome, Italy)

#### WHAT IS THE GUT MICROBIOTA?

The microbiota is «the ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our body space».

Our gut microbiota contains tens of trillion of microorganisms, including at least 1,000 different species of known bacteria with more than 3 million genes (150 times more than human genes). One third of our gut microbiota is common to most people, while two thirds are specific to each one of us.

Research suggests that the relationship between gut microbiota and humans is not merely commensal (a non-harmful coexistence), but is rather a mutualistic relationship. The microorganisms perform a crowd of useful functions, such as fermenting unused energy substrates, training the immune system, preventing growth of harmful, pathogenic bacteria, regulating the development of the gut, producing vitamins for the host, such as biotin and vitamin K, and producing hormones to direct the host to store fats. In return, these microorganisms procure within the host a protected, nutrient-rich environment in which they can thrive. However, in certain conditions, some species are thought to cause disease by producing infection or increasing cancer risks for the host.

Over 99% of the bacteria in the gut are anaerobes, but in the cecum, aerobic bacteria reach high densities. The compositions of microbiota rely on several factors like host diet, colonization history and immune status. Some microbes are better suited to complement specific metabolic enzymes than others.

#### PRODUCTION OF GAMMA-AMINOBUTYRIC ACID (GABA) IN GUT BACTERIA

Colonization of the intestine by enteric bacteria relies largely on their ability to survive in the extremely acidic environment of the stomach, the major bactericidal barrier of the gastrointestinal tract. The gut bacteria produce GABA, as part of the acid protection *gad* system. In *Escherichia coli* for instance, GABA production relies on the activities of two glutamate decarboxylase isoforms (GadA and GadB), which are the only enzymes capable of synthesizing GABA. These enzymes act in concert with the inner membrane antiporter GadC, which imports the amino acid glutamate (L-Glu) and exports GABA in the extracellular environment to protect *E. coli* from extreme acid stress (pH≤2.5).

In higher organisms, GABA is a molecule affecting many biological processes and plays a crucial role in the central nervous system by inhibiting neurotransmission.

GABA has also been recognized as an essential trophic factor during early postnatal development of the brain, as well as in adult.

Considering the current interest in how gut microbiota impacts host health and disease, exploring whether and how bacterial GABA produced by gut microbiota affects host physiology by local or long-range signaling effects is an exciting field of research.

The composition of gut microbiota is unique to each individual, just like our fingerprints !

*Escherichia coli* is a bacterium that can be found in human intestines.

The general aim of this project is to analyze the role of GABA of bacterial origin in functions that are not directly related to acid resistance.

The consortium expects that the potential outcomes of the proposed exploratory project will be (i) to identify the molecular bases of *gad*-related phenotypes important for colonization and infection, such as motility, aggregation and monoor multi-species biofilm formation (ii) to contribute to the understanding of the role of GABA levels in bacterial colonization and virulence potential in two different *in vivo* models, mouse and zebrafish and (iii) to determine to which extent GABA produced by gut bacteria can pass the intestinal barrier and potentially reach the host central nervous system, eventually influencing host behavior.

The Institut Pasteur (Paris) partner expertise on bacterial biofilm and bacteriabacteria interactions in animal models and the IPIN-Roma partner's long-standing experience on the genetic and biochemistry of bacterial *gad* system will be an asset to the implementation of the proposed research program.

# Impact of *Plasmodium/Trypanosoma* co-infections PTR INSTITUT transmit the malaria parasites



#### Project PTR n° 542-2015

Coordinator: Dr Christian MITRI, Genetics and Genomics of Insect Vector Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Brice ROTUREAU, Institut Pasteur (Paris), and Dr Mawlouth DIALLO, Institut Pasteur in Dakar

#### WHAT IS TRYPANOSOMA?

Trypanosoma is a protist parasite transmitted to humans and animals by the bite of a Glossina, more commonly known as the tsetse fly, which was previously infected from humans or animals carrying the parasites. This parasite is responsible for Human African Trypanosomiases (HAT), also known as sleeping sickness, and for Animal African Trypanosomiases (AAT), also known as nagana. These extracellular parasites first proliferate in the blood and can ultimately cross blood-brain barrier to invade the central nervous system leading to wake/sleep disorders, coma and finally death. In absence of treatment, AAT, which are highly prevalent in some African countries, cause serious economic losses in livestock, especially because untreated cases are fatal.

These parasites are present exclusively in sub-Saharan Africa. The populations most vulnerable to the tsetse fly, and therefore to the disease, are rural people who depend on agriculture. livestock and hunting.

To date, there is no vaccine, but several efficient drugs exist for humans and animals despite their reduced availability, elevated cost and severe side effects.

#### TRYPANOSOMA/PLASMODIUM CO-INFECTION

In parts of sub-Saharan Africa, the parasites responsible for malaria and trypanosomiases are transmitted sympatrically due to the presence of both insect vectors in the same ecological zones. Therefore, in these areas, the Anopheles mosquitoes, vector of Plasmodium, can bite individuals or animals carrying Plasmodium and/or Trypanosoma causing a co-infection of the vector.

#### **THE PROJECT**

Teams from the Institut Pasteur in Paris and Dakar will join their expertise in parasitology, immunology and entomology to implement this project. They want to understand how the presence of two parasites (Plasmodium and Trypanosoma) in the Anopheles (malaria vector), resulting from simultaneous or consecutive ingestions, could impact on the development and the natural transmission of Plasmodium. For this, scientists will compare data of mono-infections or coinfections, obtained in the laboratory using a mouse model as well as in the field in remote villages of Senegal. This project will improve scientific knowledge of the vectorial capacity of Anopheles subjected to at least two different parasites. These results may have an impact in terms of public health through mapping the risks of transmission and planning vector control.

Sleeping sickness threats the populations of 36 sub-Saharan countries.

65 million individuals are exposed to Trypanosoma and there are 20,000 estimated cases.

Over the last 10 years, > 70%of cases were observed in the Democratic Republic of Congo.

# Understand interactions between a protein of *Influenza* virus and host proteins to able to anticipate new pandemics



#### Project PTR n° 546-2015

**Coordinator:** Dr Caroline DEMERET, Molecular Genetics of RNA Viruses Unit - Institut Pasteur (Paris) **Institut Pasteur International Network collaborator:** Dr Sumana SANYAL, Hong Kong University - Pasteur Research Pole

#### WHAT IS INFLUENZA OR FLU?

Influenza or flu is an acute and contagious viral disease of the respiratory tracts, caused by *influenza* virus affecting the birds and some mammals, including humans and pigs. It represents a major public health problem.

It evolves as recurrent seasonal epidemics and occasional pandemics. There are three types of viruses (A, B and C), the types A and B are the most frequent. The viruses of type A are the most virulent.

The vaccination is the most effective way to prevent infection. Each year, the vaccine is updated to cover the majority of *influenza* viruses due to their permanent evolutions. Antiviral drugs exist but the virus has developed a resistance to these drugs.

#### **INFLUENZA VIRUS?**

The evolution of *influenza* virus is linked, on the one hand, to the low level of fidelity of a complex of viral proteins constituting the viral polymerase, which is involved in the replication of the viral genome. The generated viral variants are the cause of seasonal epidemics in the human population.

On the other hand, the virus evolves towards new sub-types through the exchange of viral gene segments between animal and human species, they are defined as reassortant viruses. These new viruses can cause pandemics susceptible to be devastating as the Spanish flu of 1918 causing an estimated 50 million of deaths in the human population.

#### **THE PROJECT**

The two teams of the Institut Pasteur, one in Paris and the other in Hong Kong, will associate their expertise to decipher the strategies developed by *influenza* virus to replicate efficiently in humans, and to escape the mechanisms of host defense. The work will concern particularly the interaction between the polymerase of *influenza* viruses and a specific subset of host cell proteins. It aims at deciphering the role of this interplay in the replicative viral cycle and its potential contribution to strain virulence. This knowledge will have an impact on the public health by allowing, through the identification of interactions for preventive or therapeutic purposes.

Worldwide, influenza outbreaks are responsible for 3 to 5 million cases of severe illnesses of which 250.000 to 300.000 lead to death.

In France, 2 to 7 million people are affected by influenza, every winter.

In France, the annual health and social costs for an average outbreak of flu is 460 million euros.

# Understand the origin of resistant bacterial infections in newborns in low-income countries to better fight



#### Project PTR n° 558-2015

**Coordinator:** Dr Philippe GLASER, Biology of Gram-Positive Pathogens Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Jean-Marc COLLARD and Dr Perlinot HERINDRAINY, Institut Pasteur de Madagascar and Dr Lulla OPATOWSKI, Institut Pasteur (Paris)

#### WHAT IS ANTIBIOTIC RESISTANCE?

Antibiotic resistance corresponds to a natural phenomenon of defense of bacteria towards the antibiotic treatments, which then become ineffective to fight human as well as animal infections. Recognized by the World Health Organization (WHO) as a major public health problem and a major threat for the future, antibiotic resistance is being observed in all regions of the globe. In this context, the low-income countries constitute a particularly favorable environment for the emergence and growth of multiresistant «super-bugs», because of the poverty, the weakness or the inexistence of the health care systems, the abuse and misuse of antibiotics and counterfeit drugs.

#### WHAT IS THE STRATEGY TO FIGHT AGAINST THIS TROUBLE?

It has been shown that the gut microbiota - a population of 100,000 billion microorganisms, from around 1,000 different species of bacteria essential for the digestion or the protection against infection - plays a key role in the emergence and spread of multiresistant bacteria. Yet, it is in the first days after birth, that this internal ecosystem develops under the influence of different factors still poorly known. The researchers from the Institut Pasteur in Paris and Madagascar aim to study these factors and determine how they can promote or prevent the colonization of the newborn by multiresistant bacteria.

#### **THE PROJECT**

The Institut Pasteur, and more particularly the team of Philippe Glaser, analyze the DNA from over 4,000 stool samples collected within the Institut Pasteur International Network (IPIN) and via the BIRDY project. BIRDY is a ambitious global monitoring program of infections caused by resistant bacteria in more than 10,000 infants between the birth and 18 months in low-income countries. The analysis of these samples will enable the teams to understand the reason bacteria become resistant to the most powerful antibiotics, and how they colonize the young children digestive tract. The study will contribute to finalize new prevention measures against bacterial infections among children in areas of high insecurity. It also aims to enrich our knowledge for the development of new treatments more targeted and adapted to the needs of the populations.

Antibiotic resistance is responsible for 700,000 deaths per year worldwide.

In 2050, antibiotic resistance could become a top cause of death outpacing cancer.

In the world, 200 newborns die per hour following a bacterial infection.

## Understanding the relationship between malnutrition / Pediatric Environmental Enteropathy [PTR] | PASTEUR and the immune system in low income countries to develop prevention strategies

#### Project PTR n° 08-2016

Scientific coordinators: Dr Philippe SANSONETTI and Dr Pascale VONASCH, Molecular Microbial Pathogenesis Unit -Institut Pasteur (Paris)

Institut Pasteur International Network collaborators: Dr Inès VIGAN-WOMAS, Institut Pasteur de Madagascar and Dr Matthew ALBERT, Institut Pasteur (Paris) \*

#### MALNUTRITION AND PEDIATRIC ENVIRONMENTAL ENTEROPATHY

Pediatric malnutrition in children remains a scourge in many developing countries because it is a major cause of mortality and morbidity. Despite the establishment, for several years, of prevention and control programs, chronic malnutrition still affects too many children in these countries, mainly Southern Asia and sub Saharan Africa. The lack of food or inadequate nutrition practices are however not the only causes of malnutrition. In countries with limited resources, the deteriorated sanitary and hygiene environment continuously expose children to infectious agents.

Pediatric Environmental Enteropathy (PEE) is a chronic inflammation of the small intestine, a very common syndrome in children living in countries with limited resources. PEE seems to result from continuous exposure to a heavily contaminated environment. This syndrome, which disrupts the functioning of the intestine, is now recognized as one of the major causes of malnutrition.

Malnutrition is responsible for growth retardation, reduced cognitive performance and severe learning difficulties. The first two years of a child's life is a critical period for his physical and mental development. In case nothing is done, the child never catches up with normal development and will remain affected for the rest of his life.

#### PEDIATRIC ENVIRONMENTAL ENTEROPATHY AND IMMUNE SYSTEM

PEE is a syndrome that disrupts the intestinal functions and prevents proper absorption of food. During PEE, the intestinal barrier is impaired by increased bacterial proliferation causing excessive inflammation and chronic activation of the immune system, protecting the body against infections.

It has been observed that vaccines such as those against cholera, typhoid, polio and rotavirus exhibit reduced effectiveness in children living in developing countries compared to children living in industrialized countries. The hypothesis is that PEE may be responsible for the poor efficacy of vaccines. But to date, scientific data on PEE are almost non-existent and there is no quick and easy diagnostic tool. It is important to better understand the mechanisms of this inflammation and its involvement in the alteration of the immune system to develop interventions for the benefit of children's health.

#### **THE PROJECT**

Scientists from Institut Pasteur (Paris) and Madagascar join their expertise in microbiology, immunology to implement this project. They will study systemic and mucosal immune responses in children living in Madagascar with PEE and/or chronic malnutrition. The results will be compared to those children not suffering from malnutrition. The observed immunological changes may well explain the altered response to live-oral vaccines. With this new knowledge, new intervention strategies can be developed to better protect young children against severe infections.

This project will also lead to the identification of new biomarkers of PEE, which in the long term, could be used in strategies to boost the immune system of children with PEE.

\* This project also involves / involved the participation of external partner(s).

According to WHO, the malnutrition is responsible for more than 3 millions deaths per year in children under age 5 years.

In the world, one child in four aged under 5 suffer from chronic malnutrition.

In Madagascar, 47% of children under 5 years are affected by chronic malnutrition.



# Better understanding host/virus interactions in order to develop therapeutic strategies



#### Project PTR n° 10-2016

**Coordinator:** Dr Nathalie PARDIGON, Environment and Infectious Risks Laboratory – Institut Pasteur (Paris) **Collaborators from the Institut Pasteur International Network:** Drs Nicolas WOLFF and Yves JACOB, Institut Pasteur (Paris), Dr Dimitri LAVILLETTE, Institut Pasteur of Shanghai – Chinese Academy of Sciences

#### WHAT ARE ARBOVIRUSES?

Arboviruses are viruses that belong to 5 virus families, causing diseases such as dengue, Zika, chikungunya, yellow fever and encephalitis (Rift Valley fever, Japanese encephalitis). Transmission occurs through bites from blood-sucking insects such as mosquitoes, sandflies and ticks. Infection occurs via the bloodstream, alternately from vector to vertebrate and vertebrate to vector. Humans are not the only vertebrate hosts—there are other mammals (monkeys, rodents, bats, domestic animals), birds, amphibians and reptiles.

The majority of arboviruses are essentially found in tropical areas, but their presence in a temperate region such as France is not unusual. Regions affected by arboviruses continue to expand, due to the spread of the mosquito vectors, which is favoured by the scale of international trade and travel, climate change and the formidable abilities of these mosquitoes to adapt.

Arbovirus infections cause a variety of symptoms due to the fact that they belong to different families of viruses.

Despite the existence of vaccines against some arboviruses (yellow fever, Japanese encephalitis, tick-borne encephalitis, dengue), there is no specific treatment for arboviral diseases. Prevention and control of arboviruses is based on effective measures for vector control and individual protection against mosquito bites

#### **HOST/VIRUS INTERACTIONS**

Like all intracellular pathogens, arboviruses exploit the functions of the cellular proteins of the host they infect in order to maintain their life cycle. Thus, the pathogenesis caused by viruses is mainly the result of a dysfunction in the "cellular machinery."

Many host cellular proteins have domains known as PDZs, which have been found to interact with short sequences called PBMs (PDZ binding motif) identified on viral proteins. During infection, these host/virus interactions modify the host's cellular processes for the benefit of the virus's life cycle, and may help in its spread. However, these interactions and generally the life cycle of these viruses are relatively poorly known, hence the absence of an effective treatment

#### **THE PROJECT**

The Institut Pasteur teams from Paris and Shanghai will combine their expertise in virology, structural biology and immunology to carry out this project.

They wish to study and characterise the role of PBM (virus)/PDZ (host) interactions in the life cycles of different arboviruses such as West Nile, Japanese encephalitis, dengue and Zika viruses. The study of these interactions both *in vitro* and *in vivo* could help to highlight the important sequences of the virus and host, which could be used as targets in the design of antiviral compounds specific for these arboviruses.

The results obtained will help to improve knowledge of the interactions between virus and host. The characterisation of these interactions could be a key element in the development of new therapeutic strategies

An estimated 440000-1300000 Zika virus infections have occurred in the most outbreak in Brazil.

Zika virus has been reported in more than 70 countries and territories worldwide since 2015.

There is no cure and no vaccine for Zika, Chikungunya diseases.

# Better understanding bacteria-host interactions in allergic asthma to prevent it and treat it



#### Project PTR n° 18-2016

Scientific coordinator: Dr Anne TSICOPOULOS, Center for Infection and Immunity – Institut Pasteur in Lille (France) Institut Pasteur International Network collaborators: Dr Mathias CHAMAILLARD, Institut Pasteur in Lille (France), Dr Ivo GOMPERTS-BONECA, Institut Pasteur (Paris)

#### WHAT IS ASTHMA?

Asthma is a chronic lung disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person. During an asthma attack, the lining of the bronchial tubes swell, causing the airways to narrow and reducing the flow of air into and out of the lungs. The strongest risk factors for developing asthma are a combination of genetic predisposition with environmental exposure to inhaled substances and particles that may provoke allergic reactions or irritate the airways like tobacco smoke, air pollution, allergens (for example: mold spores, pollen, house dust mites). The symptoms are chest tightness, wheezing, shortness of breath and coughing.

Asthma is not just a public health problem for high income countries: it occurs in all countries regardless of level of development. Over 80% of asthma deaths occurs in low and lower-middle income countries. Urbanization and climate change have been associated with an increase in asthma.

Allergic asthma is the most common type of asthma.

#### ALLERGIC ASTHMA AND IMMUNE SYSTEM

In allergic asthma, among breathed allergens, house dust mite represents the most common cause of this disease. It has been shown the house dust mites contain some bacterial components.

When these components enter into a contact with cells of organism, they are detected as foreign bodies by the immune system cells. The recognition involves receptors expressed by cells of immune system. These receptors include NOD-like Receptor protein 1 (NOD-1) that recognizes microbial patterns.

Currently, the mechanisms of the immune response involving allergen and NOD-1 are not clearly defined.

#### **THE PROJECT**

The scientists from Institut Pasteur Paris and Lille will combine their expertise in immunology and microbiology to analyze the mechanisms involving lung immune responses and allergens. For this, they will define how NOD-1 sensing of dust mite may promote allergic asthma. First, these studies will be done in vitro, then they will assess the relevance of these observations *in vivo* with a mouse model relevant to human disease.

This project should unveil how allergic response to house dust mite is regulated by NOD-1 and pave the way towards new therapeutic strategies and open new perspectives on prevention of the burden of asthma in the next future. 235 million people suffer from asthma in the world. It is the most common chronic disease among children.

In France, asthma affects more than 4 million people, one third of whom are under the age of 15.

The economic costs of asthma are high (treatment, hospitalization, reduced activity levels, school and working absenteeism).

# Better understanding of the mechanism responsible for chronic Hepatitis B to better diagnose and treat



#### Project PTR n° 20-2016

**Scientific coordinator:** Dr Maryline BOURGINE, Molecular Virology and Vaccinology Unit - Institut Pasteur (Paris) **Institut Pasteur International Network collaborators:** Dr Silvia PICONESE, Institut Pasteur – Cenci Bolognetti Foundation (Rome) and Dr Yu WEI, Institut Pasteur (Paris)/Institut Pasteur of Shanghai - Chinese Academy of Science \*

#### WHAT IS HEPATITIS B?

Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV) that affects the liver. It is one of the most prevalent human diseases.

It can cause both acute and chronic disease. Acute hepatitis B is often asymptomatic. Some people do not recover from hepatitis B and they develop a chronic infection associated with hepatic lesions of variable severity. This chronic infection can progress to cirrhosis and liver cancer (hepatocellular carcinoma). Most chronic carriers do not present obvious symptoms, despite showing signs of liver inflammation and be able to contaminate their immediate circle.

The virus is transmitted by exposure to infected blood and various body fluids, usually via sexual contact, blood transfusions and from mother to child at birth.

Treatments for chronic infection can slow progression to cirrhosis, reduce incidence of liver cancer and improve long term survival but they do not cure hepatitis infection. Vaccination against hepatitis B virus is the primary reliable measure to protect people.

#### INFLAMMATION, IMMUNE RESPONSE AND INFECTION

Inflammation is the body's protective response against infection. This is a complex process involving various types of immune cells, coagulation proteins and signaling molecules, which evolves over time. Inflammation plays a critical role in tissue repair by eliminating injured cells through the action of immune cells, which are controlled by secretion of mediators. Highly coordinated interactions between immune cells and secreted mediators guarantee efficient development and resolution of the inflammatory response and restoration of tissue homeostasis. In contrast, uncontrolled and excessive inflammation produces pathologic effects.

In viral infections such as Human Immunodeficiency Virus and Hepatitis C Virus, a mechanism involving specific immune cells was found responsible for the maintenance of chronic immune activation. Does this mechanism play a role in the state of chronic inflammation in HBV infection?

#### **THE PROJECT**

The three teams involved in this project will combine their expertise in immunology and virology to understand cellular mechanisms responsible for maintaining inflammation during chronic hepatitis B. The scientists will study two cell populations from the immune system in patients with HBV infection at different stages of chronic disease, and also in mouse models of chronic HBV infection with and without liver inflammation. These studies will allow to evaluate the contribution of these two cell populations to the outcome of the HBV specific cell responses in a context of chronic hepatitis B infection and hepatic inflammation. These new knowledge would pave the way for the identification of novel biomarkers for prognostic and diagnostic of chronic HBV infection and for the generation of innovative therapeutic approaches.

\* This project also involves / involved the participation of external partner(s).

In the world, 2 billion people have been infected by hepatitis B virus and over 370 million are chronic carriers.

In 2015, hepatitis B resulted in 887,000 deaths (including cirrhosis and hepatocellular carcinoma).

In France, over 3 million people have been a contact with HBV, 300,000 are chronic carriers.

## Better understanding of the invasive capabilities of bacteria in order to develop new drugs and assist in diagnosis



#### Project PTR n° 22-2016

Scientific coordinator: Dr Nathalie SAUVONNET, Molecular Microbial Pathogenesis Unit – Institut Pasteur (Paris) Institut Pasteur International Network collaborator: Dr Priscille BRODIN, Institut Pasteur in Lille

#### WHAT ARE SHIGELLA FLEXNERI AND MYCOBACTERIUM TUBERCULOSIS?

Shigellosis is an acute infection of the intestine caused by *Shigella* sp. (such as *S. flexneri*). Symptoms include fever, nausea, vomiting, and diarrhea that is usually bloody. Diagnosis is clinical and confirmed by stool culture. Antibiotics are given to moderate to severely ill patients with bloody diarrhea, immunocompromise and young children. However, frequent antibiotic resistance strains are now reported.

Tuberculosis (TB) is caused by bacteria, *Mycobacterium tuberculosis* that requires high levels of oxygen and most often affect the lungs. TB is spread from person to person through the air. People infected with TB bacteria have a 10% lifetime risk of falling ill with TB. However, persons with compromised immune systems, such as people living with HIV, malnutrition or diabetes have a much higher risk of failing ill. The major symptoms associated with active lung TB are cough, fever, fatigue, weight loss/anorexia, chest pains. TB is treatable, curable and preventable disease. But there is emergence of drug resistance strains.

# INTEREST OF STUDY MODELS (STUDY MODELS TO DECIPHER INVASION OF PATHOGENS)

In infections by *S. flexneri* and *M. tuberculosis*, the tissues particularly exposed to these pathogens are respectively the gut and the lung. These tissues have a multicellular organization that is constantly exposed to mechanical forces (pressure, shear stress and other forces due to peristaltic contraction or breathing effort).

Despite an increasing amount of knowledge on the molecular machineries elaborated by pathogens to manipulate the host cells, a lot of questions on the very early steps of invasion at the tissues level are still unanswered due to the methodological limitation.

To understand and visualize the infection of these two bacteria in a physiological context, it is necessary to develop a new system biomimicking human functional organ.

#### **THE PROJECT**

Scientists from Institut Pasteur Paris and Lille decided to bring together their forces to overcome to the methodological limitations for the study of first invasion steps of pathogens. For this, they are going to use a new technology called «organ on a ship» recapitulating the complex structure and function of an organ. They will design and make «gut-on-a-ship» and «lung-on-a-ship» and these devices will enable them to study the effect of the physiological and mechanical constraints on *S. flexneri* and *M. tuberculosis* respectively in intestine and lung.

This project will allow to advance the scientific knowledge in the invasion mechanism and pathogenicity of bacteria at the tissue level. These developed systems will offer new capacities to test the efficiency of new identified drugs.

Shigellosis kills from 700,000 to 1 million people each year in the world.

In 2015, 10,4 million people fell ill with **TB** and 1,8 million died from the disease.

Globally in 2015, an estimated 480,000 people developed multidrug-resistance TB.

# To better understand the evolution of pathogenic bacteria in order to develop new therapeutic strategies



#### Project PTR n° 24-2016

Scientific coordinator: Dr Bianca COLONNA, Laboratory of Molecular Microbiology - Institut Pasteur – Cenci Bolognetti Foundation (Rome, Italy) Institut Pasteur International Network collaborator: Dr Didier MAZEL, Institut Pasteur (Paris) \*

#### WHAT ARE SHIGELLA AND ENTEROINVASIVE E. COLI?

Shigella and Enteroinvasive E. coli (EIEC) are very similar types of bacteria that cause intestinal infections.

Shigellosis is an infectious disease caused by a group of bacteria called *Shigella*. *Shigella* is extremely contagious and the exposure to a very small amount can cause illness. People infected with bacteria release it into their stool. They can spread the bacteria to water or food or directly to another person. The symptoms are diarrhea (often blood), fever, stomach cramps and pain. Outbreaks of Shigellosis are linked with poor sanitation, contaminated food and water and crowded living conditions. This illness is common among infants and children in countries with poor general hygiene conditions, travelers in developing countries and residents in refugee camps.

*Enteroinvasive Escherichia coli* is a type of pathogenic bacteria whose cause syndrome identical to Shigellosis with diarrhea and fever. The causes of EIEC infection are contaminated food, soil or water with human feces. These infections are treated with antibiotics but some strains develop resistance to the antibiotics.

#### **GENOME ORGANIZATION OF BACTERIA SHIGELLA AND EIEC**

The genome of these bacteria is consisted by a unique chromosome that carries all the genes needed by the bacterium for its survival and development. Beside the chromosome, these bacteria contain always a large plasmid (circular DNA molecule) which carries most of the genes encoding proteins required for the invasive process and in particular those encoding a peculiar molecular syringe able to inject the so-called invasins directly into the host cells. The pathogenicity process depends on the presence of this plasmid.

What are the relationship between the chromosome and the plasmid? How do these elements contribute to the emergence of new strains causing severe infections?

#### **THE PROJECT**

The scientists from Institut Pasteur of Rome and Paris will combine their expertise (molecular genetics and genomics) to tackle several questions concerning the genetic and relationships between the virulent phenotype and the genome arrangement of *Shigella* and EIEC. For this, they will sequence different bacterial strains, and will compare the genome of different strains in order to define the evolution of the bacterial genetic organization and the relevance to virulence gene expression. This knowledge will help to understand the genetic and molecular mechanisms governing the stability of virulence plasmids in these bacteria and, in future, will contribute to the design of new therapeutic strategies.

\* This project also involves / involved the participation of external partner(s).

Shigellosis is held responsible for some 165 million cases of severe dysentery, the majority in developing countries and involve children less than 5 years of age.

More than one million people are estimated to die from *Shigella* infection each year.

Some 580,000 cases of Shigellosis are reported among travellers and military personnel from industrialized countries.

# Study Aspergillosis in Cambodia to investigate the impact of this pathogen in East Asia



#### Project PTR n° 26-2016

Scientific coordinator: Dr Jean-Paul LATGE, Aspergillus Unit – Institut Pasteur (Paris) Institut Pasteur International Network collaborator: Dr Alexandra KERLEGUER, Institut Pasteur in Cambodia \*

\* This project also involves / involved the participation of external partner(s).

#### **ASPERGILLUS - ASPERGILLOSIS**

Aspergillosis is an infection or allergic response caused mainly by *Aspergillus fumigatus*. The natural habitat of the fungus is the soil where it grows on dead leaves, compost piles or in other decaying vegetation. The spores of this fungus are in the air around us and we are constantly breathing them. While, they are totally harmless for most people, they can cause various forms of mycosis. But in immunocompromised patients or patients with lung disease, they are responsible of fungal pulmonary pathologies. The major forms of diseases are aspergilloma, allergic pulmonary aspergillosis, chronic pulmonary aspergillosis and invasive aspergillosis.

The symptoms depend on the type of infection. The treatment is based on the use of corticosteroids (aerosol or oral administration) for allergic patients or antifungal medicines in association with steroid therapy.

#### **ASPERGILLOSIS IN CAMBODIA**

Accurate clinical and epidemiological data regarding the incidence of invasive fungal infections from developing regions of the world (e.g. Asia-Pacific) is meagre due to the fact that these countries do not have the economic resource for such studies.

To date, nothing has been published regarding the incidence of aspergillosis within the immunocompromised and immunocompetent population in Cambodia. This is surprising, considering the vital association of aspergillosis with AIDS, cancer, and other underlying respiratory disorders.

These high incidence rates of aspergillosis in countries lying in proximity to Cambodia underscores the importance of conducting surveillance and gathering epidemiological data for this country. Initiating aspergillosis research in Cambodia will be very important to bridge the current gap in knowledge and provide essential data for appropriate medical care.

#### **THE PROJECT**

The major objective of this project is to establish an *Aspergillus* research group at the Institut Pasteur of Cambodia in order to study Aspergillosis in this country. The development of this structure will be possible thanks to a collaborative work between a team from Institut Pasteur Paris and another one from Institut Pasteur of Cambodia. Firstly, they will develop new methods of diagnosing Aspergillosis to efficient screen the population who are clinically asymptomatic for aspergillosis. Then, they will isolate and characterize *Aspergillus* strains from clinical samples to create a database and collate epidemiological data related to mycoses. They will test the antifungal susceptibility and elucidate the prevalence of resistance for Cambodian strains.

This knowledge can help to implement sustainable public health directives in Cambodia. They will also pave the way for better surveillance and management of this disease within the Cambodian population.

Over 300 million people are acutely or chronically infected by fungi, leading to death, long term illness, blindness, reduced work capacity.

The major chronic, invasive and allergic forms of aspergillosis account for around 600,000 deaths annually worldwide.

Chronic pulmonary aspergillosis accounts for 3 million cases in the world.

## Study the *Negativicutes* to better understand the involvement of a poorly characterized component of the microbiome in human health



#### Project PTR n° 39-2016

Scientific coordinator: Dr Simonetta GRIBALDO – Molecular Biology of Gene in Extremophiles Unit – Institut Pasteur (Paris)

Institut Pasteur International Network collaborators: Dr Christophe BELOIN, Institut Pasteur (Paris) and Dr Maria L. BERNARDINI, Institut Pasteur – Cenci Bolognetti Foundation (Rome)

#### WHAT ARE NEGATIVICUTES?

The *Negativicutes* are anaerobic bacteria that are present in the normal oral and intestinal microbiome of humans.

Many members of the *Negativicutes* inhabit the human environment, with the bestknown being *Veillonella*. This bacterium has a dual role, beneficial or harmful. They have in fact been described as the earliest colonizers of the gut of infants and premature babies and may therefore participate in the maturation of the innate immune system. However, they can also transform into opportunistic pathogens, and have been isolated from the mucosa-associated microbiota and saliva of patients with Crohn's disease.

#### **PARTICULARITY OF NEGATIVICUTES**

The *Negativicutes* have a particular cell envelope, which includes an outer membrane with LPS (lipoPolySaccharide), a well-known immuno-modulator. This is unusual, as the *Negativicutes* belong to the classical Gram-positive *Firmicutes*. The existence of didermic cell envelopes in the *Firmicutes* is little known by the scientific community and represents an evolutionary puzzle. Moreover, the role of *Veillonella* in health and disease has not been studied.

#### **THE PROJECT**

The scientists of this project will combine their expertise to obtain first data on the role of *Negativicutes* in the human microbiome by using *Veillonella* as a model. They will characterize the LPS of *Veillonella* and study its interaction with the immune system. Moreover, they will analyze the role of cell envelope structures in the community behavior of *Veillonella* with other common bacterial residents of the human microbiome.

This project will bring new data concerning an understudied component of the human microbiome and opportunistic pathogen. It could reveal some important aspects of the link between *Negativicutes* and the development of a healthy immune system, as well as their role in gut homeostasis or dysbiosis.

Many bacteria of the human microbiome are still poorly studied.

The *Negativicutes* may have a dual role, beneficial and pathogenic.

This project will provide important information for the role of the microbiome in health and disease.

# A better understanding of the immune system for improved patient care



#### Project PTR n° 35-2016

**Scientific coordinators:** Dr Aissatou TOURÉ – Immunology Unit – Institut Pasteur in Dakar and Dr Darragh DUFFY, Immunobiology of Dendritic Cells Unit – Institut Pasteur (Paris)

**Institut Pasteur International Network collaborators:** Dr Milena HASAN, Dr Anavaj SAKUNTABHAI, Dr Magnus FONTES and Dr Alexandre ALANIO, Institut Pasteur (Paris) and Dr Cheikh LOUCOUBAR and Dr Muriel VRAY, Institut Pasteur in Dakar

#### WHAT IS THE IMMUNE SYSTEM?

The immune system is a complex system that protects the body against infectious organisms such as bacteria, viruses, parasites and fungi and other foreign invaders. The immune response attacks organisms and substances that invade the body and cause disease.

The immune system is made up of a network of cells, tissues, and organs that work together to protect the body. There are different types of cells involved in the immune response that work together to recognize antigens (foreign substances that invade the body) and respond. Each type of cells has separate functions such as engulfing organisms or remembering and recognizing previous invaders to help the body to destroy them or produce antibodies, which are specialized proteins that lock onto specific antigens.

#### WHAT IS THE VARIABILITY OF THE IMMUNE RESPONSE ?

Susceptibility to infections, severity of disease and response to drugs and to vaccines are highly variable from one individual to another. Inter-individual variability has long been neglected in medical practice and public health policies, where a single approach to disease management and drug development are common.

New vaccines against infectious disease are often developed and tested in western populations who have a different genetic background, are in contact with highly divergent sets of infections and have different dietary practices and nutritional conditions. As such, efficacy rates are known to drop significantly upon clinical testing in non-US / non-European populations.

It is necessary to have a better understanding of how genetic and environmental differences ((e.g., local endemic infections (latent infections, commensal microbial communities) and lifestyle behaviours (dietary practices)) impact immune responses.

In this context, the Institut Pasteur (Paris) teams launched a few years ago a project called Milieu Intérieur funded by « Laboratoire d'Excellence » call. This project is studying 1,000 healthy individuals from France to understand and define the response variability of the immune system at the population level and to determine the genetic and environmental factors involved. This project is a multidisciplinary study involving teams from immunology, microbiology, virology, mathematics and genetic backgrounds.

Now these scientists want to combine their expertises with those teams from Institut Pasteur in Dakar to expand the geographical reach and ethnic diversity of the project. The current project in Senegal proposes a pilot study with 48 donors who will be recruited in two villages with different genetic background and ecosystems. It will benefit from the tools developed within the « Milieu Intérieur » project.

One important objective of this project in Senegal is to evaluate the feasibility and requirements of a larger scale study in this country.

This project is poised to have a strong impact on public health, as the data generated will greatly improve our understanding of individual variability in immune responses.

It will deliver unprecedented information for the development of new vaccines, therapies, and *in vitro* diagnostics for improved patient management. As such it will improve the effectiveness and efficiency of future public health initiatives in both Northern and Southern countries.

# Characterize the role of ExoY, virulence factor, in *Pseudomonas aeruginosa* infections to identify therapeutic strategies



#### Project PTR n° 43-2016

Scientific coordinator: Dr Undine MECHOLD, Biochemistry and Macromolecular Interactions Unit- Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Stoyanka STOITSOVA, Stephan Angeloff Institute (Bulgaria), Dr Lhousseine TOUQUI, Institut Pasteur (Paris) \*

#### WHAT ARE PSEUDOMONAS INFECTIONS?

*Pseudomonas* infections are diseases caused by a Gram-negative bacterium from genus *Pseudomonas*. The bacteria are found widely in the environment, such as in soil, water and plants. Only a few of the many species cause disease. The most common species that causes infection is called *Pseudomonas aeruginosa*.

They usually do not cause infections in healthy people. However, healthy people can develop mild illnesses, especially after exposure to water. More severe infections occur in people who are already hospitalized with another illness, called nosocomial infections, or people who are immunocompromised, such as HIV or AIDS patients, people receiving chemotherapy, patients with cystic fibrosis.

Infections can occur in any part of the body as blood, lungs, skin, ear and eye. Symptoms depend on which part of the body is infected. Pseudomonas infections can be very aggressive, particularly infections in the lungs or skin.

Pseudomonas infections are treated with antibiotics. Unfortunately, these bacteria have developed resistance to the treatment. These infections could be fatal in people who are already very ill.

#### **VIRULENCE FACTORS AND PSEUDOMONAS AERUGINOSA**

Virulence factors are molecules expressed and secreted by the bacteria. They play an important pathological role in the colonization, the survival of the bacteria and the invasion of tissues and causing damage to the host.

The pathogenicity of *P. aeruginosa* relies on a complex arsenal of soluble factors (toxins, enzymes,...) and cellular structures (pili, secretion systems,...) having complementary effects. *Pseudomonas aeruginosa* is able to inject into the host cells toxic effectors (virulence factors) via a secretion system (T3SS). Currently, four toxic effectors have been identified including ExoY. The gene expressing ExoY is present in 90-98% of clinical isolates suggesting an important role in pathogenesis. In agreement with this, ExoY activity was lately associated with severe damage to lung tissue suggesting ExoY as potential drug-target in the treatment of *P. aeruginosa* infections. Therefore, its role needs to be deepen.

#### **THE PROJECT**

Teams from Institut Pasteur Paris and Stephan Angeloff Institute having complementary expertise will associate to characterize the role of the ExoY in *P. aeruginosa* infections and also study the toxicity of this toxin in the host cells. This project will allow to develop a cell line that will be used to study the effects of this toxin in the cell and also to identify specific inhibitors of ExoY or its activation.

This new knowledge will permit to explore therapeutic approaches for the treatment of acute or/and chronic infections associated to cystic fibrosis disease and acute nosocomial infections induced by *P. aeruginosa* in immunocompromised patients.

\* This project also involves / involved the participation of external partner(s).

*Pseudomonas aeruginosa*, third cause of nosocomial infections in France.

In the patients with cystic fibrosis, the bacteria contribute to the decline of the respiratory function.

About 60% of people with cystic fibrosis have a chronic respiratory infection caused by *P. aeruginosa*.

# Better knowledge of nAChRs and identification of new ligands for therapeutic purposes



#### Project PTR n° 03-2017

Scientific coordinator: Dr Pierre-Jean CORRINGER, Channel Receptors Unit – Institut Pasteur (Paris) – CNRS UMR 3571 Institut Pasteur International Network collaborators: Dr Rym BENKHALIFA, Institut Pasteur in Tunis, Dr Pierre LAFAYE (Antibody Engineering Platform) and Dr Uwe MASKOS, Institut Pasteur (Paris)

#### WHAT ARE THE NICOTINIC ACETYLCHOLINE RECEPTORS?

Nicotinic acetylcholine receptors (nAChRs) are receptor proteins that are activated by the neurotransmitter, acetylcholine. They act as ion channels, eliciting cellular excitation when open. They are localized in the central and peripheral nervous system and also in muscles. The nAChRs are the key players of the neuronal communication in the brain and at the neuromuscular junction.

They are composed of 5 identical or homologous subunits, generating a typical pentameric « rosette » crossing the membrane of cell. Each subunit is composed of two domains: an extra-membrane domain carrying the binding site for acetylcholine and a trans-membrane domain with a channel. When acetylcholine binds to the site of the extra-membrane domain, this leads to a conformational change and in a few hundred of microseconds the channel opens and releasing a signal in the brain. Numerous nAChRs subtypes exist from the combinatorial association of a repertoire of 16 subunits.

They are also involved in the control of voluntary movements, memory and attention, sleep and waking, pain and anxiety. The dysfunction of these nAChRs is associated with schizophrenia, Alzheimer's disease and stroke. They are also involved in myasthenic pathologies and tobacco addiction.

#### NICOTININC ACETYLCHOLINE RECEPTORS AND LIGANDS

The nAChR present different binding sites for the ligands, orthosteric site (primary site) and non-orthosteric sites (non-conventional sites that are distinct from the orthosteric site). These receptors can bind acetylcholine and also toxins of snakes and scorpions or nicotine. The ligands have different effects on the receptors like agonists, inhibitors, or allosteric modulators...

Over the past decades, research has focused mainly on the orthosteric site which is well conserved among the various nAChRs. The identified ligands however present weak specificity among the different nAChR subtypes. Interestingly, few molecules targeting other binding sites have been described, although they likely will display better specificity and milder effects.

#### **THE PROJECT**

The scientists of this project from IP Paris and IP Tunis gather together their expertises (nicotinic receptors biochemistry, electro-physiological experiments, nanobodies production and histology) to develop novel classes of small protein ligands able to bind to nAChRs. For this, they are focusing on 3 nAChRs to identify ligands from scorpion toxins and single–domain of antibodies called nanobodies. These ligands, will be expected to target both orthosteric and non-orthosteric sites. Once the identified ligands, the scientists are going to characterize them, identify the locus of binding site and study their effects on the nAChR functions. The discovered toxins and nanobodies will allow studying the nAChR in vitro and *in vivo*, and will also all development of tests to screen for small molecule ligands with therapeutic potential. They will open for the future the way for the drug design approaches.

Schizophrenia, mental disorder, that affects more than 21 million people in the world.

Alzheimer's disease in France: 900,000 patients, 225,000 new cases diagnosed per year.

The tobacco epidemic kills more than 7 million people each year worldwide.

# Understand how the ubiquitination regulates functions of HP1, a protein involved in ageing and several pathological conditions including cancer



#### Project PTR n° 24-2017

Scientific coordinator: Dr Giovanni CENCI – Department of Biology and Biotechnology "C. Darwin" - Institut Pasteur - Cenci Bolognetti Foundation (Rome, Italy) Institut Pasteur (Paris) collaborator: Dr Christian MUCHARDT, Institut Pasteur (Paris)

#### WHAT IS HETEROCHROMATIN PROTEIN 1 (HP1)?

The family of Heterochromatin protein 1 (HP1) consists of highly conserved eukaryotic proteins, which have important functions in the cell nucleus. These proteins play fundamental roles in chromatin architecture, gene expression silencing and telomere homeostasis. There are three HP1 proteins family members HP1 $\alpha$ , HP1 $\beta$  and HP1 $\gamma$  encoding for their own gene in humans, respectively CBX5, CBX1 and CBX3.

The structure and function of CB5, CBX1 and CXN3 genes are highly homologous from Drosophila to humans allowing studies to be performed both in Drosophila and in mammalian cells.

Studies in Drosophila and mammalian cells have also indicated that HP1 plays a pivotal role in telomere homeostasis and protection. HP1-dependent heterochromatin organization is essential for longevity in flies.

In human cells, the role of HP1 during aging has mostly been studied in the context of senescence, a cellular manifestation of aging. In particular, senescence of human cells is associated with mislocalization of HP1 proteins and altered chromatin compaction. The multiple functions of the HP1 are likely to be correlated with the many post-translational modifications described for these proteins.

#### **HP1 AND UBIQUITINATION**

Proteomic studies have shown that Drosophila HP1 as well as the three mammalian HP1 proteins are ubiquitinated. The ubiquitination is the "kiss of death" process for a protein. In ubiquitination, a protein is inactivated by attaching ubiquitin to it. Ubiquitin is a small molecule. It acts as a tag that signals the protein-transport machinery to ferry the protein to the proteasome for degradation.

Despite it is largely known that HP1 undergoes a variety of post-translational modifications that influence its activity, the role of the ubiquitination is still elusive. Indeed experiments in mice have shown that ubiquitination is affected upon ageing and in neurodegenerative disorders involving protein aggregation.

#### **THE PROJECT**

Two teams from IP Rome and IP Paris will combine their expertise to understand how ubiquitination may regulate HP1 functions in telomere maintenance, gene expression and replicative senescence. In this proposal they will undertake a synergic approach to unravel the contribution of HP1 ubiquitination at both cellular (human and Drosophila cell lines) and organismal (Drosophila) levels. They will first carry out a proteomic analysis (Mass Spectrometry) in order to determine what residues get ubiquitinated and then to identify conditions resulting in modified ubiquitination of HP1. Then they will assess the role of the HP1 ubiquitination on telomere homeostasis and cellular senescence.

The results of this project will provide new insights in the comprehension on the epigenetic mechanisms preventing genome instability onset that underlies ageing and several pathological conditions including cancer. Epigenetic regulator of cellular ageing.

# Better understanding of genetic epidemiology and pathogen evolution of *Leptospira* will improve diagnostic and prevention



Scientific coordinator: Dr Mathieu PICARDEAU – Biology of Spirochetes Unit – Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Julien GUGLIELMINI, Institut Pasteur (Paris), Dr Fréderic VEYRIER, INRS-Institut Armand Frappier (Canada), Dr Cyrille GOARANT, Institut Pasteur in New-Caledonia, Dr Nikolay TOKAREVICH, Institut Pasteur in St Peterburg (Russia), Dr Alejandro BUSCHIAZZO, Institut Pasteur in Montevideo, Dr Anissa Amara KORBA, Institut Pasteur in Algeria \*

#### WHAT IS LEPTOSPIROSIS?

Leptospirosis is a neglected infectious disease caused by bacteria belonging to the genus *Leptospira*. Leptospirosis can be transmitted to humans through the contact of abraded skin or mucous membranes with water that is contaminated with urine of infected animals, such as rodents. Rodents are considered the main reservoirs. Leptospirosis also affects the livestock, causing abortions, infertility and loss of milk production in cattle, this represents a serious economic problem.

Leptospirosis is a complex disease with nonspecific clinical manifestation and difficult diagnosis. In humans, Leptospirosis can cause a wide range of symptoms including high fever, chills, headache, muscle aches, similar to many other diseases, such as dengue fever and malaria.

This disease occurs worldwide but is found predominantly in impoverish populations inhabiting developing countries with tropical climate. Leptospirosis is expected to become more important in Europe due to global climate changes and the rapid urbanization. Outbreaks can occur following excessive rainfall or flooding.

#### **A LARGE DIVERSITY OF STRAINS**

*Leptospira* are ubiquitous organisms that are found as free-living saprophytes (microorganism that lives on dead organic matter) in environmental water and soil or as pathogens that can cause infections (from asymptomatic carriage in rats to lethal acute infection in both humans and animals). Nevertheless, little is known about of the agents of leptospirosis, which are difficult to isolate from biological samples. *Leptospira* presents a high variability of the genus, consisting of 22 species and more than 300 serovars (variation within *Leptospira* species). But the data available in public databases represent a tiny proportion of this genetic heterogeneity due to the complexity of *Leptospira*. Furthermore, virulence evolution has been under-explored, providing little information regarding the virulence factors and their roles in the pathogenesis due to the absence of genetic tools.

Better characterization of *Leptospira* serovars is absolutely necessary to understand the epidemiology of leptospirosis.

#### **THE PROJECT**

The scientists from different Institut Pasteur around the world bring together their expertise to understand global and local phylogeographic trends, but also genetic factors that are associated with disease severity and reservoir specificity, using their recent advances in genetics. They are going to create a unique collection of over 1,000 *Leptospira* strains from different geographical origins and hosts that will constitute the most important and representative collection in the world.

They are going to characterize the strains from this collection by sequencing the genome, identify mutations. They will compare all these information in order to understand the origin and genome evolution of pathogenic *Leptospira* and will also identify new candidates for virulence factors.

Knowledge will allow to better understanding of leptospiral pathogenetic mechanisms and evolution of *Leptospira* strains and they will contribute to design new diagnostic tests and vaccines in the future.

This project also involves / involved the participation of external partner(s).

Leptospirosis is a zoonotic and emerging bacterial disease.

Leptospirosis cause more than 1 million severe cases with 60,000 deaths per year.

In Europe, the highest incidence is observed in France (600 cases per year).



# Development of a new genetration of antituberculosis vaccines for preventive and therapeutic approaches



#### Project PTR n° 52-2017

Scientific coordinator: Dr Marta ROMANO, *In vivo* models Unit / Immune Response Service – Scientific Institute of Public Health (Brussels, Belgium) Institut Pasteur (Paris) collaborators: Dr Laleh MAJLESSI and Dr Pierre CHARNEAU \*

#### WHAT IS THE TUBERCULOSIS?

Tuberculosis (TB) is an infectious disease caused by bacteria, mainly *Mycobacterium tuberculosis* (*Mtb*), that most often affects the lungs. *Mtb* is an airborne pathogen that is spread through the air from pulmonary TB patients before onset of an effective drug therapy.

Infection with *Mtb* does not always result in active TB disease. Indeed, the vast majority of infected individuals is able to control the infection to an asymptomatic level, this is defined as latent *Mtb* infection (LTBI). In LTBI, the bacteria remain in the body in an inactive state because the immune system stops them from spreading. People have no symptoms and are not contagious but reactivation of a latent *Mtb* infection can occur. In active TB, the bacteria multiply in the body and in the case of pulmonary TB can be transmitted to other people. The symptoms include cough, fever, night sweats, weight loss, coughing up blood or sputum.

TB affects all age groups and all parts of the world. However, people with compromised immune systems are most at risk of developing active TB.

The majority of TB cases can be cured when the right medication is available and administered correctly, with antibiotics taken for a relatively long time and a treatment success rate of 83%. However, some strains have become resistant to the available standard drugs. In this case, active TB can be treated with the use of very specific anti-TB drugs, but the duration of treatment considerably increase and its success rate falls to 52%.

A preventive TB vaccine (bacille Calmette-Guerin or BCG) is used for infants and newborn children in many countries.

#### **BCG - CURRENT ANTI-TUBERCULOSIS VACCINE**

The BCG, created in 1921 by A. Calmette and C. Guerin (in IP Lille), is currently still the only vaccine administered at birth for TB prevention in endemic countries. However its efficacy remains controversial because it confers a highly variable protection against pulmonary TB in adults (from 0 to 80%). This poor efficacy can be explained by different factors (i) the waning of immune responses induced by vaccination at birth over time (ii) BCG is a poor inducer of protective cellular immune responses and responses specific to antigens detected in Latent TB (iii) BCG does not induce responses specific to numerous antigens potentially contributing to protection.

Several vaccines have been developed in the last three decades either to replace BCG (live attenuated vaccines) or as booster vaccins in adulthood (subunit vaccines). Although some have reached clinical evaluation, their improved efficacy is not guaranteed.

The design of vaccine candidates must therefore continue in order to achieve development of a new generation efficient TB vaccine.

TB is still among the 10 top causes of death worlwide.

It is estimated that about a third of the world's population are infected with *Mycobacterium tuberculosis*.

Three teams from SIPH (Brussels) and IP Paris are going to associate their complementary expertise (Immunology, vaccinology, TB and lentiviral vectors) in order to develop better preventive and therapeutic approaches against human TB. For this, they will investigate in the mouse and rat TB models three promising new live attenuated vaccine candidates combined to non integrative lentiviral vectors encoding multiple TB antigens as boosters. Lentiviral vectors are gene transfer vectors derived from HIV. They are strictly non-replicative since they are deleted of all HIV coding sequences, but express genes of interest, such as genes of selected antigens. Lentiviral vectors have a strong tropism for dendritic cells *in vivo*, and thus allow the use a the endogenous pathway of Ag presentation. This translates into the induction of intense and long lasting cellular immune responses.

In this collaborative projects, antigens will be selected among highly immunogenic TB proteins and multi-antigenic lentiviral vectors will be designed. These vectors will be tested in combination with three live-attenuated vaccine candidates as booster vaccines following priming with live-attenuated vaccines. Induced vaccine-responses and protective efficacy against infection with *Mtb* will be performed in both mouse and rat for comparison purposes. Moreover, immune therapeutic potential of multi-antigenic LV vectors in combination with anti-mycobacterial treatment will be analyzed.

This project will generate data which will contribute to the development of next preventive and therapeutic approaches for TB.

In 2016, 6.3 million new cases of TB were reported.

\* This project also involves / involved the participation of external partner(s).

# Understand how Leptospires escape the human immune system in order to develop new therapeutic and vaccine strategies



#### Project PTR n° 66-2017

Scientific coordinator: Dr Catherine WERTS, Innate Immunity and Leptospira Group, Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Mariko MATSUI, Institut Pasteur in New Caledonia, Dr Jessica QUINTIN, Institut Pasteur (Paris) \*

#### WHAT ARE LEPTOSPIRA INTERROGANS?

*Leptospira interrogans* are zoonotic bacteria responsible for leptospirosis, a neglected reemerging disease.

Rodents such as rats and mice are asymptomatic carriers of *Leptospira* in their kidneys, and excrete them in the urine, which contaminates the environment and other animals. Pathogenic leptospires are able to evade the host complement system, circulate and replicate in blood and spread into tissues.

Humans develop a mild to severe acute disease potentially fatal, due to multiorgan failure. Human leptospirosis is often diagnosed late, due to large spectrum of symptoms. The disease can be treated with antibiotics that are effective when started early after infection.

The available vaccines are inactived leptospires confering short lasting protection, restricted to only one serovar.

#### LEPTOSPIRA AND THE IMMUNE SYSTEM

Microbes are recognized by phagocytes (such as macrophages, neutrophils and dendritic cells) through Pattern Recognition Receptors (PRR) including the Toll-Like Receptors (TLR) and Nod-Like Receptors (NLR). These receptors are cellular signaling receptors playing a decisive role in pathogens' recognition and alerting the immune system. Following invasion, *leptospires* are mostly detected by TLR2, the receptor of lipoproteins. In human cells, the leptospiral LPS (present in the outer membrane of bacteria) activates TLR2, but not TLR4, the receptor of classical LPS. In the mouse model, the leptospiral LPS is recognized by both TLR 2 and 4. The TLR4 recognition causing an inflammatory response was shown protective in the mouse model. *Leptospires* also escape the human and mouse NOD receptors recognizing cell wall components.

#### **THE PROJECT**

Three teams of Institut Pasteur Paris and in New Caledonia will combine their expertise in the fields of leptospirosis and immunology to understand whether macrophages, dendritic cells and neutrophils fulfill or not their respective protective roles in leptospirosis.

This proposal is based on the parallel study of mouse and human phagocytes responses toward leptospirosis. The use of TLR4 deficient mice or humanized mice that mimic the human recognition, will help us to understand which phagocytic responses are deficient, and potentially how to restore them using TLR and NLR agonists.

This project of fundamental research will generate novel scientific knowledge that should lead to new therapeutic or vaccine strategies.

\* This project also involves / involved the participation of external partner(s).

How *leptospires* escape from phagocytes?

Use TLR and NOD agonists to restore phagocyte functions?

# A comprehensive understanding on the nitransport pathways to enhance the current therapeutic strategies in *helicobacter pylori* infections



#### Project PTR n° 73-2017

Scientific coordinator: Dr Charles CALMETTES, Microbiology Department - INRS - Institut Armand Frappier (Canada) Institut Pasteur International Network collaborators : Dr Hilde DE REUSE, Institut Pasteur (Paris), Dr Frédéric VEY-RIER, INRS – Institut Armand Frappier (Canada)

#### WHAT ARE HELICOBACTER PYLORI?

*Helicobacter pylori (H. pylori)* is a gram-negative bacteria that causes chronic inflammation in the inner lining of the stomach (gastritis) and in duodenum in humans. This bacterium is also considered as a common cause of ulcers worldwide. *H. pylori* often infects the stomach during childhood. This infection spreads from person to person by saliva, fecal contamination (in food or water) and poor hygiene practices.

*H. pylori* adapt to live in the harsh, acidic environment of the stomach by generating substances that neutralize stomach acids. This makes the stomach cells more vulnerable to the harsh acids. Stomach acid and *H. pylori* together irritate the stomach lining and may cause ulcers in the stomach and duodenum.

Most people with *H. pylori* infection do not have any symptoms. When the infection leads to an ulcer, symptoms may include abdominal pain, heartburn, excessive burping, feeling bloated nausea, lack of appetite.

People infected with *H. pylori* have an increased risk of stomach cancer. Experts estimate that 1 to 3% of the infected population will develop gastric carcinoma, a pathology with poor prognosis (15% survival 5 years after prognosis) representing the third most common cause of death by cancer.

The diagnosis of *H. pylori* infection includes tests for antibodies in blood, a urea breath test, tests for antigens in stools and endoscopic biopsies.

The standard treatment includes a combination of two antibiotics with a proton pump inhibitor (PPI) that reduces acid production by the stomach. The efficiency of the treatment decreases due to *H. pylori* strains resistant to antibiotics. A new medication that combines two antibiotics, PPI and a metal, bismuth (bactericidal activity), has proven high efficacy in *H. pylori* treatment.

#### **NI-TRANSPORT IN HELICOBACTER PYLORI**

*H. pylori* is the only bacterium that can multiply in the stomach, a hostile acid niche. In *H. pylori*, Nickel (Ni) is of extraordinary importance as it is a genuine virulence determinant. Ni is co-factor of two enzymes that are indispensable for *in vivo* colonization : (FeNi)-hydrogenase and the very abundant urease, a major virulence factor that is essential for *H. pylori* resistance to gastric acidity.

The Ni concentration in the human body is very low. Thus, a constant and important supply of nickel is required for the survival of *H. pylori* within the stomach, implying a tight control of its acquisition, distribution and storage. Important players of Ni-transport have been identified but their structures, precise mode of action, their interplay and additional partners are yet to be discovered. These transporters are also involved in uptake of bismuth, a metal used in *H. pylori* therapy, since humans have no reported Ni-enzymes, essential Ni-transporters constitue very promising therapeutic targets for *H. pylori* eradication.

Helicobacter pylori infects about 50% of the world's population.

*H. pylori* is the most common cause of gastric ulcers and gastritis.

*H. pylori* is the main cause of gastric cancers.

Three scientific teams of the Institut Armand Frappier and the Institut Pasteur Paris will combine their strongly complementary expertise (bacterial evolutionary, structural biology and molecular biology) to characterize the Ni-transporters and to establish a complete picture of Ni-transport in *H. pylori*. Therefore, they will search for new partners implicated in Ni-homeostasis in *H. pylori* and in gastric Helicobacter species, and will establish their evolutionary history. They will also determine the crystallographic structures of Ni-trafficking proteins. And then, they will do the functional characterization of the multiple Ni-transporters in *H. pylori*.

The generated knowledge will be beneficial for the large community studying metal trafficking in bacterial, archaeal and eukaryotic organisms. And also, this project will have an therapeutic application by promoting medical improvement of bismuth based therapeutics.

Gastric carcinoma is responsible of 800,000 deaths a year in the world.

# Identify how the infants acquire a dysbiotic gut microbiota and PEE in the context of chronic malnutrition to develop preventive strategies



#### Project PTR n° 91-2017

Scientific coordinators: Pr Philippe SANSONETTI and Dr Violeta MOYA-ALVAREZ – Molecular Microbial Pathogenesis Unit - Institut Pasteur (Paris)

Institut Pasteur International Network collaborators: Dr Serge-Ghislain DJORIE, Institut Pasteur in Bangui, Dr Tamara GILES-VERNICK and Dr Sean KENNEDY (Biomics Platform), Institut Pasteur (Paris)\*

#### MALNUTRITION AND PEDIATRIC ENVIRONMENTAL ENTEROPATHY

Malnutrition remains a major health problem despite enormous progress in recent years, mainly in sub-Saharan Africa and Asia. Young children (between 0 to 5 years) in developing countries are the most vulnerable. Malnutrition has a direct impact on physical and psychological development of a child. The first 1,000 days of the child's life are the most decisive because malnutrition can impose irreversible consequences on his growth, immunological and neurocognitive development.

It has been reported that malnutrition in early life is often associated with a syndrome called Pediatric Environmental Enteropathy (PEE) that is generally considered as an important etiological factor, even though the causality link remains to be established. PEE encompasses changes in the resident gut microbiota, particularly a dysbiosis called small intestinal bacterial overgrowth (SIBO) that is suspected to cause chronic low grade inflammation and atrophy of the small intestinal mucosa, eventually leading to nutrient malabsorption, and deficient responses to live-oral vaccines. PEE is considered an emerging issue in public health in Sub-Saharan Africa and in South-East Asia. In the framework of the AFRIBIOTA project, Institut Pasteur teams and their collaborators are currently identifying the microbial species that compose the dysbiotic SIBO and decipher its pathogenic potential on intestinal physiology.

#### **AIM OF THE PROJECT**

The aim of MITICA is to identify the conditions in which SIBO is acquired by infants. More precisely, we will analyze if is it transmitted by the mother at birth (fecal, vaginal origin), or during the breast-feeding period, or after weaning through exposure to a massively contaminated environment carried by vectors such as food and water.

#### **THE PROJECT**

For this project, the Institut Pasteur scientists in Paris will team up with colleagues from IP Bangui and from the Henri Izamo Maternity in Bangui (Central African Republic) to identify how infants acquire the dysbiotic microbial community that constitutes the SIBO, eventually leading to PEE. Therefore, they will define the composition of the gut microbiota of malnourished and control mother-infant pairs from birth to 6 months. They will also determine breast-milk composition and they will investigate the nutritional status of mothers and infants. This study will be carried out in the context of an anthropological study aimed at developing a rich ethnographic description of concepts and practices associated with pregnancy, childbirth and newborn care among women in Bangui.

The MITICA study is leading the drive towards improving public health, enhancing treatment options for afflicted children, and understanding the biological aetiology of PEE.

\* This project also involves / involved the participation of external partner(s).

Malnutrition causes the death of 3 to 5 million children under 5 every year in the world.

Childhood stunting affects 162 million children under the age of 5 years in the world

The Mitica study will offer new solutions to prevent and treat the deleterious effects of PEE

## Develop rapidly tools for diagnostics of emerging diseases through the use of new technologies (nanobodies, droplet based microfluidics)



#### Project PTR n° 98-2017

Scientific coordinator: Dr Pierre LAFAYE - Antibody Engineering Platform - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Pierre BRUHNS, Institut Pasteur (Paris), Dr Otto PRITSCH, Institut Pasteur in Montevideo

#### HOW TO DEAL WITH AN EMERGING OR REMERGING DISEASE

Emerging and remerging infectious disease epidemics pose a significant global threat. Pathogens circulate rapidly due to globalization of travel and trade, and climate change contributes to the spread of vector-borne diseases to previously non-endemic areas.

Over the past decade, Ebola, Middle East Respiratory Syndrome (MERS), pandemic influenza and the Zika virus have each demonstrated the extraordinary health, economic and security risks associated with infectious disease outbreaks. In order to improve preparedness and reduce the negative impact of epidemics, we must answer quickly in terms of prevention, diagnosis and treatment. Regarding the diagnosis, it is necessary to rapidly develop scalable technologies for efficient and affordable diagnostic tools of different pathogens that can be used anywhere, by anyone, and at any time to screen patients. For this, research on the development of new diagnostic tools must begin now in order to be reactive during the next viral outbreak.

#### WHAT TECHNOLOGY TO USE?

Currently, most diagnostic tests use mice monoclonal antibodies but these antibodies might be not stable enough to support the rugged conditions observed in the field conditions. An alternative is to use nanobodies.

The nanobodies (or VHHs) are antibodies devoid of light chains presenting antigenbinding domain and produced by camelids (camels, dromedaries, llamas, alpacas). These nanobodies are well expressed in microorganisms and have a high stability and solubility. They recognize the antigen with a high affinity. Nanobodies obtained after immunizing alpacas, are selected by phage display. This process is laborious and time-consuming.

Droplet-based microfluidics may be the solution to increase efficiency, reduce time and costs to identify potent nanobodies. This process has been used as proof-ofconcept to antibody-producing B cell analysis and identification in immunized mice.

#### **THE PROJECT**

The scientists from Institut Pasteur Paris and Institut Pasteur in Montevideo bring together their expertise (nanobodies, microfluidics and biophysics) to develop a droplet-based microfluidic solution for high-throughput identification of antigen-specific nanobodies. This process will enable highly efficient and rapid identification of antigen-specific VHH repertoires. By using EBOLA soluble Glycoprotein (sGP) as antigen and a proof-of-concept, the applicants anticipate to discover nanobodies against sGP with diagnostic potential for Ebola Virus Disease. These VHHs could be used to develop a rapid dipstick test (EBOLA sGP ELISA test) and be tested with human samples in collaboration with Pasteur Institutes of Dakar (Senegal) or Conakry (Guinea).

With this proof-of-concept, the applicants will be able to develop faster diagnostic tools for different pathogens during emerging epidemics.

The annual global cost of moderately severe to severe pandemics is roughly \$570 billion, or 0.7% of global.

Rapid development of tools for diagnostics.

# Understand the molecular mechanisms of premature ageing in syndromes such as Cockayne syndrome to develop therapeutic strategies



#### Project PTR n°111-2017

Scientific coordinator: Dr Shahragim TAJBAKHSH - Stem Cells and Development Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators : Dr Houda YACOUB-YOUSSEF, Institut Pasteur in Tunis, Dr Miria RICCHETTI, Institut Pasteur (Paris) \*

#### WHAT IS COCKAYNE SYNDROME?

Cockayne syndrome (CS) is a rare genetic disorder, mainly characterized by small stature, microencephaly, developmental delay, impairment of vision and hearing, neurological impairment, muscle atrophy, and premature ageing. Most affected individuals have an increased sensitivity to sunlight (photosensitivity). This syndrome was described for the first time in 1936 by Dr. Cockayne.

CS has been divided into three subtypes based on the severity and age of onset of symptoms (Type I: classic form, Type II: much more severe form and Type III: mildest symptoms).

The main cause of CS is mutation in ERCC6 or ERCC8 gene responsible for the production of CSB or CSA protein, respectively. These proteins are involved in repairing damaged DNA caused by ultraviolet (UV) rays from the sun and toxic chemicals. In CS patients, DNA damage is not repaired normally and this defect has been suspected to be the main cause of premature ageing. However recent discoveries, largely from one consortium member, unveil other possible causes for this dramatic disease.

Cockayne syndrome is quite apparent right from the birth with characteristic symptoms such as small head circumference. A genetic test looking for mutation of the CSA or CSB gene confirms the diagnostic of CS.

The life expectancy of CS patients is estimated at 12 years because no treatment exists to date.

#### **MOLECULAR MECHANISMS OF PREMATURE AGEING**

Since its discovery, CS was classified as a DNA repair disease given that mutations identified in patients, are present in the CSA and CSB genes, encoding proteins involved in the DNA repair system. However, it is difficult to attribute the severe neurodegenerative damage observed in CS patients to the single failure of the DNA repair system for induced UV damage. Furthermore, mutations in the CSA and CSB genes have also been identified in patients with another syndrome known as UVSS (UV-sensitive syndrome). This is a DNA repair disease that does not predispose to neurodegenerative damage. Finally, CS patients with no photosensitivity have been described.

It is therefore essential to understand molecular mechanisms involved in this premature ageing pathology.

Incidence of CS about 1/200000 in European countries.

No prevention strategy or known cure for CS.

\* This project also involves / involved the participation of external partner(s).

Scientists from Institut Pasteur Paris and Tunis associate their complementary expertise to better characterize Cockayne syndrome in Tunisian patients and also identify some factors involved in the mechanism of ageing. For this, they will work in collaboration with different hospitals in Tunis, collaboration initiated in a previous ACIP project, to collect blood, muscle and skin biopsies from CS patients and aged persons. The collection of muscle from CS patients is unique to this project. CS patients in Tunisia have a distinct set of mutations and include extremely rare cases with little or no photosensitivity, which are precious controls to establish the causes of precocious ageing independently of the DNA repair defect. Indeed, in a previous study, one of the consortium members identified a defective mitochondrial function in fibroblasts from progeroid patients, which is independent of the DNA repair defect. Therefore, they will explore in different types of cells (skin, blood), and for the first time a tissue rich in mitochondria (muscle), from the same CS patients whether the mitochondrial function is globally defective, thereby demonstrating a new mechanism involved in CS. In addition, they will assess key mitochondrial factors in muscle cells from young and aged human individuals and mice, in order to identify whether there are common regulators between premature and normal ageing. Normal human ageing is characterized by muscle loss, called sarcopenia, whose causes have not been identified. Finally they will investigate the role of a pharmacological agent (MnTBAP) on muscle regeneration in young and aged individuals and mice. They previously discovered that this molecule is able to revert the mitochondrial dysfunction in fibroblasts of CS patients and it was recently designated as Orphan Drug by the European Medecine Agency for use in Cockayne Syndrome. The comparative analysis of accelerated and normal ageing should thus help to identify unique and common features of both conditions in order to develop therapeutic strategies that can be used for premature and normal ageing.

The number of people aged 60 years or older will rise from 900 million to 2 billion between 2015 to 2050 (moving from 12% to 22% of the total global population).

# Understand the role of a new family of lymphocytes in colorectal cancer to develop prevention and therapeutic strategies



#### Project PTR n° 113-2017

Scientific coordinators: Dr Angela SANTONI and Dr Giuseppe SCIUME, Department of Molecular Medecine - Institut Pasteur - Cenci Bolognetti Foundation (Rome, Italy) Institut Pasteur (Paris) collaborators: Dr James DI SANTO and Dr Christian VOSSHENRICH \*

#### WHAT IS COLORECTAL CANCER?

Colorectal cancer (CRC) is cancer of the large intestine (colon), which is the final part of he digestive tract. Most cases of CRC begin as a benign tumor, often in the form of a polyp, which over time becomes cancerous.

Most CRC are due to old age and lifestyle factors with only a small number of cases due to underlying genetic disorders. Some risk factors include diet (low fiber, high-fat), obesity, smoking and lack of physical activity. Another risk factor is inflammato-ry bowel disease, which includes Crohn's disease and ulcerative colitis.

Signs and symptoms may include blood in the stool, a change in bowel movements, unexplained weight loss and weakness or fatigue.

CRC diagnosis is performed by sampling of areas of the colon suspicion during coloscopy and it is confirmed by microscopical examination of a tissue sample.

Treatments used for CRC include some combination of surgery, radiation therapy, chemotherapy, immunotherapy.

In some cases, it is possible to make lifestyle changes to reduce the risk of CRC by engaging in physical activity, consuming a diet high in fiber and reducing smoking and alcohol consumption. Screening (eg. coloscopy or fecal occult blood test) has the potential to reduce CRC.

#### **COLORECTAL CANCER AND INNATE LYMPHOID CELLS (ILCS)**

CRC is usually infiltrated by immune cells involved both in the innate and acquired immune responses.

Recently, a group of lymphocytes has been associated with CRC, playing a key role in the initiation and progression of gut inflammation. These cells are Innate Lymphoid Cells (ILCs), a newly discovered class of innate immune cells that are also involved in regulation of gut microbial homeostasis, immune defense and tissue repair. They react promptly to signals from infected and injured tissues producing cytokines. These cells are divided in 3 groups (ILC1, ILC2 and ILC3) based on the cytokines that they produced. ILCs do not express antigen receptors and are activated by stress signals, microbial coumpounds and cytokines.

Experiments in mouse models have allowed to observe dysregulated cytokine expression in the local environment. However, the molecular mechanisms that link ILC function and CRC development are not yet identified. Thus, identification of novel cellular and molecular pathways involved in the regulation of the physiological balance of pro-inflammatory cytokines is a pressing unmet need.

Colorectal cancer is a global threat with more the 1 million of new cases and more than 700,000 deaths each year.

Colerectal cancer is the fourth cause of tumor death worldwide.

Colorectal cancer is the second most common cancer in women and third in men.

\* This project also involves / involved the participation of external partner(s).

Two teams from Institut Pasteur in Paris and Rome will combine their strong complementary expertise, in basic fundamental studies, clinical translational research and innovative mouse models, to understand the role of ILCs and the molecular pathways associated with CRC. For this, they will focus more specifically on ILCs expressing receptors named Natural Cytotoxicity Receptors (NCR) and transcription factors belonging to the family of Signal Transducer and Activator of Transcription (STAT). Therefore, they will understand the role of ILCs expressing these receptors and transcription factors in the pathogenesis of CRC and they will also define the expression of cytokines in the intestinal environment during CRC development. These studies will be performed using different mouse CRC models and on human biological samples from patients with CRC.

This study will provide (i) novel knowledge into pathways involved in CRC pathogenesis and (ii) new paths to design both preventive and therapeutic strategy for CRC.

About 54% of colorectal cancer cases occurred in more developed countries.

## Better understanding of genetic diversity and epidemiology of Enteroviruses in Central Africa and in Madagascar in order to improve the surveillance



#### Project PTR n° 161-2019

Scientific coordinator: Dr Maël BESSAUD, Viral populations and Pathogenesis Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Ionela GOUANDJIKA-VASILACHE, Institut Pasteur in Bangui, Dr Serge-Alain SADEUH-MBA, Pasteur Centre in Cameroon, Dr Richter RAZAFINDRATSIMANDRESY, Institut Pasteur in Madagascar \*

#### WHAT ARE ENTEROVIRUSES ?

Human infections by enteroviruses (EVs) are very common, particularly during childhood. Tens of EV types are known to be able to infect humans that are responsible of a high number of infections worldwide.

The vast majority of known members of the *Enterovirus* genus mainly replicate in the gut or in the respiratory tract. Enteric EVs are resistant to acid pHs which allows them to travel through the stomach to reach the gut and they are mainly excreted in stools. Respiratory EVs are acid-sensitive and they are transmitted through cough and sneeze.

In the vast majority of cases, EV infections remain asymptomatic or trigger very mild symptoms. Nonetheless, in some cases, acute EV infection can degenerate into severe manifestations such as poliomyelitis, encephalitis, myocarditis, respiratory diseases that can be fatal or induce long-lasting sequelae.

#### **GENETIC DIVERSITY OF EVS**

In previous works, the scientists of this project have highlighted a high genetic diversity among the EV ecosystems in central Africa and in Madagascar, mainly promoted by frequent genetic exchanges between co-circulating EVs. Such exchanges are probably favoured by poor sanitation conditions, which increase the rate of human coinfections. Moreover, they identified virus types and lineages that have never been identified elsewhere. Therefore, central Africa and Madagascar appear to host two specific EV ecosystems made of both viruses that circulate worldwide and viruses whose circulation seems to be restricted to central Africa and/or to Madagascar. Yet, these works were affected by two limitations: 1) focus on EVs excreted in stool samples and thus did not consider EVs that mainly replicate in respiratory tract, 2) use established cell lines to isolate EVs from stool samples and thus missed non-cultivable EVs and EVs that do not induce cytopathic effect in cell lines.

It has been known for decades that some EV types do not grow or do not produce clear cytopathic effects on cell lines used for EV diagnosis or surveillance. New screening methods based on molecular assays and/or deep sequencing have revealed a part of the EV ecosystem that was previously hidden.

Stools and nasopharyngeal swabs constitute best choice samples for such investigations since they contain relatively high concentration of EVs. Wastewater samples ideally complement clinical samples since they concentrate EVs excreted by many people and even by wild and domestic animals. Wastewater samples were shown to not only contain enteric EVs but also EVs displaying respiratory tropism.

EV 71 (EV-A71) is a type commonly associated with severe neurological complications during hand, foot, and mouth disease outbreaks, particularly in South-eastern Asia.

Recently, EV D68 (EV-D68) emerged as the causative agent of sporadic but severe respiratory disease outbreaks across the United States, Asia, Africa, and Europe.

\* This project also involves / involved the participation of external partner(s).

Scientists from 4 institutes of Institut Pasteur International Network (Paris, Bangui, Cameroon and Madagascar) will combine their expertise to identify the mechanisms that generate genetic diversity in EV ecosystems and to understand how this diversity promotes the emergence of new virus lineages with modified phenotypic properties.

For that, they will use new technologies to uncover the parts of the EV ecosystems that have remained hidden during their previous works, to study the epidemiology of these viruses and to decipher the genetic links they have with other EVs. For this purpose, they plan to perform direct screening of RNA extracted from clinical samples (nasopharyngeal swabs and stool samples) and wastewater samples collected in Cameroon, Central African Republic and in Madagascar by using EV-targeting molecular assays. RNA extracted from positive samples will be subsequently used to directly amplify EV genomes and to sequence them using next-generation sequencing technology. Phylogenetic studies will be conducted to determine how close viruses that circulate in the two considered regions are to viruses identified elsewhere. Because recombination is an important driver of EV evolution, they will also study whether the new populations of EVs uncovered by this work exchange or not genetic sequences with EVs classically observed with standard techniques. They will also detect, quantify and characterize recombination by-products that lead to the generation of defective viral particles whose role in the evolution of EV populations remains unknown.

These results will throw new light on the genetic diversity of EVs that infect humans in geographic regions with specific ecosystems and also will provide valuable information about the epidemiology of the EVs. These information will help to conceive future studies to determine the impact of these EVs on human health where they circulate.

# A better knowledge of the CyaA toxin at the structural level will allow therapeutic and vaccinal improvements



#### Project PTR n° 166-2019

Scientific coordinator: Dr Alexandre CHENAL, Biochemistry of Macromolecular Interactions Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Gérard PEHAU ARNAUDET (UTechS Ultrastructural Biolmaging), Dr Ahmed HAOUZ (Crystallography Platform), Dr Patrick ENGLAND (Molecular Biophysics Platform), Sébastien BRIER (Biological NMR Technological Platform) and Dr Olivier SPERANDIO, Institut Pasteur (Paris), Dr Joo Hwan NO, Institut Pasteur in Korea

#### WHAT IS WHOOPING COUGH?

Whooping cough is a very contagious respiratory disease caused by the bacterium *Bordetella pertussis*.

People with whooping cough usually spread the disease by coughing or sneezing. Many infants with whooping cough are infected by parents and older siblings. Whooping cough is a serious disease that can cause infants to stop breathing. Pregnant women need to be vaccinated against whooping cough and those surrounding infants need to keep updated their whooping cough vaccination.

Prevention is mainly by vaccination with the pertussis vaccine. Initial immunization is recommended between six and eight weeks of age, with four doses to be given in the first two years of life.

Most deaths occur in young infants who are either not vaccinated or partially vaccinated.

Antibiotics may be used to prevent the disease in those who have been exposed and are at risk of severe disease.

#### THE ADENYLATE CYCLASE (CYAA) TOXIN

The adenylate cyclase (CyaA) toxin is one of the major virulence factors secreted by *Bordetella pertussis*. CyaA plays an important role in the early stages of respiratory tract colonization by *B. pertussis*.

The molecular mechanisms involved in the CyaA toxin translocation through the plasma membrane of eukaryotic target cells remain largely unknown. CyaA carries its own translocation machinery, and it is the only known toxin reported to date able to invade cells by a direct translocation of its catalytic domain (ACD) from the extracellular environment to the cytosol (i.e. the liquid phase inside the cells) across the plasma membrane of the eukaryotic target cells. The translocated ACD of CyaA (post translocation state) binds calmodulin (the ACD eukaryotic effector) and produces supraphysiological levels of cAMP (considered as second messenger) that ultimately lead to cell death and subvert host defense.

Although CyaA has been extensively studied at the cellular level and engineered for vaccinal purposes, structural characterization of the full-length toxin has been hampered thus far by its poor solubility. CyaA is a large multi-domain protein, which exhibits a pronounced hydrophobic character, making it prone to aggregation into non-functional forms. Very recently, scientists of this consortium achieved to refold CyaA into a monomeric and fully functional form, stable in the absence of any chaotropic agents. This opens new perspectives toward in-depth biophysical and structural characterization of this complex toxin.

Worldwide, it is estimated that approximately 15 million pertussis cases and about 200 000 pertussis deaths occur per year.

The CyaA toxin constitutes a potent non-replicating vector to deliver antigens into antigen presenting cells and induce specific cell mediated immune responses.

This project is an integrative structural biology project bringing together scientists of the Institut Pasteur in Paris and in Korea with complementary expertise (biochemistry, biophysics, crystallography, ultra-structural bioimaging, HDX-MS, bio informatic). The aim of this project is (i) to discover inhibitors of ACD-calmodulin activity by small molecule screening, (ii) to solve the structure of CyaA by a combination of X-ray crystallography and electron microscopy (cryo-EM), and (iii) to gain new insights into the toxin inserted into membranes as well as its effect on the membrane properties by a combination of methods (including various biophysical approaches, such as CD, fluorescence, FTIR, cryo-TEM, HDX-MS, FRET, biolayer interferometry, microscale thermophoresis, ...).

From an applied perspective, this knowledge will pave new ways to improve (i) treatments of whooping cough, and the use of CyaA (ii) as a protective antigen for pertussis vaccination and (iii) as antigen delivery vector for therapeutic vaccines.

# Development of a new highly sensitive diagnostic test for sleeping sickness to improve surveillance in the context of HAT elimination

#### Project PTR n° 175-2019

Scientific coordinator: Dr Brice ROTUREAU, Trypanosome Transmission Group - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Lucy GLOVER, Dr Marie-Noelle UNGEHEUER and Albane IMBERT, Institut Pasteur (Paris), Dr Noël TORDO, Institut Pasteur in Guinea \*

#### WHAT ARE TRYPANOSMES?

African trypanosomes are parasites transmitted by the bite of the tsetse fly. Wild and domestic animals can host these parasites and may represent an important reservoir of infection for the tsetse flies in several transmission foci.

In human, they cause the debilitating neglected tropical disease, sleeping sickness or Human African Trypanosomiasis (HAT).

The parasites, *Trypanosoma brucei gambiense*, responsible for 98% of human cases, first reside in the patient blood and skin for months to years before invading the central nervous system, where they cause the neurological symptoms of the disease.

The specific drug and treatment course will depend on the type of infection and the disease stage.

No effective vaccine currently exists.

#### **DIAGNOSTIC TOOLS**

HAT was included in the World Health Organization (WHO) roadmap on neglected tropical diseases, with 2020 set as target date for elimination as a public health problem. A secondary goal of zero transmission by 2030 has also been set. These targets have, in part, been encouraged by the success of surveillance efforts that rely on detecting extracellular trypanosomes in human blood. Nevertheless, the reduction in case numbers brings about other challenges. For example, the sensitivity of any diagnostic test diminishes as the disease burden drops, and this is being seen with the serological tests available for HAT. In this context, new highly sensitive and specific diagnostic tools will be required to accurately monitor the occurrence of new cases and the possible emergence of drug-resistant trypanosomes during the elimination phase.

Most diagnostic tests currently under development are based on optimization of existing methods that may not combine all the requirements to stand up to the harsh constraints imposed by the elimination phase requirements, especially in terms of sensitivity and specificity.

#### THE PROJECT

Scientists from Institut Pasteur in Paris and in Guinea will combine their expertise to adapt the recently developed Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK) technology that combines a CRISPR-Cas system and lateral flow test to the detection of trypanosomes. This will provide the sensitivity and specificity required for a diagnostic test in the elimination and post elimination phases.

Furthermore, SHERLOCK reaction reagents can be lyophilized for cold-chain independence and longterm storage and be readily reconstituted on paper for field applications. A lateral flow test for a simple and rapid readout can be easily implemented after a reaction that does not exceed two hours from the sampling step.

They propose to adapt and optimize a new SHERLOCK approach for the detection of trypanosome-specific nucleic acid sequences in human body fluids. Then, they will validate it application using fresh samples from experimentally infected mice and archived or fresh samples from patients with HAT.

In total, this project would provide an ideal field-adapted method for the diagnosis of HAT during the elimination and post-elimination phases.

\* This project also involves / involved the participation of external partner(s).

Although over 57 million people are currently at risk of contracting HAT in rural sub-Saharan Africa.

The number of cases reported in 2017 has dropped to approximately 1,442 from only a dozen African countries.

The Democratic Republic of Congo still accounts for more than 80% of these remaining cases and the Republic of Guinea hosts the most active transmission foci in Western Africa.


## Validation of a next generation diagnostic assay for antimicrobial resistance detection in order to enhance personalized treatment of MDR-TB patients



#### Project PTR nº 183-2019

Scientific coordinator: Dr An VAN DEN BOSSCHE, Bacterial Diseases Unit - Sciensano (Brussels, Belgium) Institut Pasteur International Network collaborators: Dr Roland BROSCH, Institut Pasteur (Paris), Dr Alain BAULARD, Institut Pasteur in Lille and Dr Igor MOKROUSOV, Institut Pasteur in St Peterburg (Russia) \*

#### WHAT IS TUBERCULOSIS

Tuberculosis (TB) is caused by infection with the bacterium *Mycobacterium tuberculosis* (Mtb) and predominantly affects the lungs, resulting in extensive tissue pathology. Most infections are asymptomic, which is defined as latent tuberculosis. About 10% of latent infection progress to active TB that, if left untreated, kills about half of those affected. The classic symptoms of active TB are a chronic cough with blood containing mucus, fever, night sweats and weight loss. Tuberculosis spreads in the air when people with active TB cough, spit, talk or sneeze. People with latent TB do not spread the disease.

The disease, which is particularly widespread in the developing world comprising 30 high-burden countries, is one of the top 10 causes of death worldwide and is the leading agent among lethal infectious diseases (ranking above HIV/AIDS). Treatment requires the use of multiple antibiotics over a long period of at least 6 months. Moreover, antibiotic resistance is a growing problem with increasing rates of multiple drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) reported. This resistance significantly prolongs treatment time to at least two years and lowers the success rate of therapy from 82% to 55%.

Tuberculosis prevention and control efforts rely primarily on the vaccination of infants and the detection and appropriate treatment of active cases.

#### **ANTIBIOTIC RESISTANCE AND DIAGNOSTIC**

Antibiotic resistance in *M. tuberculosis* emerges through mutations in drug targets and/or activation mechanisms, often but not always enabling prediction of drug resistance from its genetic code (DNA).

The roll-out of targeted amplification diagnostics, that are able to detect these specific drug resistance causing mutations, has strongly increased TB diagnostic capacity worldwide. Although these tests are fast and sensitive, they (a) do not target second-line and new drugs, giving them limited use in the treatment of MDR-TB, (b) do not distinguish living from death cells in clinical samples, (c) ignore all intrinsic resistance mechanisms like efflux pump overexpression, (d) cannot detect persister cells which display non-heritable drug resistance and (e) ignore the multifactorial influence of compensatory mutations.

To address limitations with current methods for TB drug susceptibility testing, scientists of Institut Pasteur's in Belgium (named Sciensano) and France (Lille and Paris) designed a next-generation diagnostic test which is able to perform ultra-fast phenotypic predictions of TB drug resistance based on RNA quantification. (This program was co-funded by Sciensano and ACIP program (ACIP 03-2016)).

According to the 2018 WHO report, one-third of the world's population is infected with Mtb, inflicting 10 million new TB cases each year and leading to 1.6 million deaths, despite the existence of curative chemotherapy and widespread BCG vaccine campaigns.

It is estimated that in 2017 about 558 000 people developed rifampicin resistant TB. 82% of these people were even suffering from multi-drug resistant-TB. Almost half of the cases are found in three countries: India, China and the Russian Federation.

For this project, scientists from Institut Pasteur in Belgium and France (Lille, Paris) will join their experiences with those of their colleagues from St Petersburg to fully unlock the potential of their diagnostic platform. For that, they will first expand the test to include the new TB drug-candidate macozinone. Next, they will optimize test parameters for each selected drug-bug combination. Subsequently, they will perform a interlaboratory validation of the optimized assay in a high incidence MRD-TB country (Russia). Finally, they will explore the potential of the platform to enhance TB diagnostic research.

For many decades, the battle against TB has been of paramount importance in the Institut Pasteur International Network. This innovative RNA-based approach for ultra-fast phenotypic drug susceptibility testing can greatly enhance personalized treatment of critical MDR-TB patients, and will be embraced by both the TB community and the industry. The therapy for TB cases caused by these resistant strains is long, highly toxic and has uncertain efficacy with mostly only 55% treatment success.

## Components of the protein synthesis machinery of trypanosomatids as targets for novel therapeutic strategies



#### Project PTR n° 190-2019

Scientific coordinator: Dr Beatriz GUIMARAES, Structural Biology and Protein Engineering Laboratory - FIOCRUZ (Brazil) Institut Pasteur (Paris) collaborators: Dr Sylvie POCHET and Dr Nadia IZADI-PRUNEYRE

#### WHAT ARE TRYPANOSOMATIDS?

Trypanosomatids are a group of protozoan parasites that includes the genera *Trypanosoma* and *Leishmania* which are responsible for numerous diseases of humans and animals.

The three major human diseases caused by trypanosomatids are African trypanosomiasis (sleeping sickness), American trypanosomiasis (Chagas disease) and leishmaniasis, which remain a major world health problem.

Sleeping sickness is caused by *Trypanosoma brucei*, transmitted by tsetse flies and considered endemic in 36 countries of sub-Saharan Africa.

Chagas disease is caused by *Trypanosoma cruzi* that is primaly transmitted by triatomine bugs in endemic countries. Other ways of transmission are blood transfusion, organ transplantation, as well as congenital and oral transmissions. Chagas disease is endemic in 21 countries across Latin America and patient numbers are growing in non-endemic, developed countries.

Different forms of Leishmaniasis are caused by various species of *Leishmania* parasites, which are transmitted by phlebotomine sandflies. Main forms are cutaneous leishmaniasis, which may be mutilating and disfiguring and visceral leishmaniasis, which is systemic and mostly lethal if left untreated. Leishmaniasis affects poor populations of over 100 countries across Asia, East Africa, South America, and the Mediterranean region.

Currently, there are chemotherapeutic treatments of these diseases but no vaccine for prevention.

#### TREATMENT

The treatments, despite being relatively effective, are associated with a wide list of problems, including high cost, length, duration of treatments along with severe side effects. In addition, the mechanisms of action of most compounds are unknown. These facts underline the need for the development of more specific compounds against these parasites. These are important reasons justifying the search for new drugs that differentially target trypanosomatids. Despite recent efforts, outcomes of small-molecule screens have not yet met the expectations of revealing new inhibitors with less side effects.

Recently, scientists of this project have proposed a new route to identify targets aiming at the development of selective trypanosomatid inhibitors. This route focuses on protein synthesis machinery, which are essential for cell viability. The protein synthesis machinery of trypanosomatids presents several key differences as compared to mammalian cells that can be explored to design specific inhibitors.

The main points to explore for new specific inhibitors are the detailed characterization of the regions mediating the interaction between the translation initiation factors EIF4E and the mRNA cap-4 structure, the initiation factor EIF4G and the polyadenylate-binding proteins (PABPs). Development of inhibitors directed to these binding sites are hampered by the lack of structural data describing the molecular contacts involved in these complexes.

The estimates for Human African Trypanosomiasis indicate 69 million people at risk in 36 sub-Saharan countries with approximately 3000 new cases per year reported in recent years, although the actual number of cases may be much higher.

Chagas Disease: approximately 6-7 million people infected, with 70 million at risk.

Three teams of two institutes of the IPIN (Fiocruz and Institut Pasteur Paris) will combine their complementary expertise to study the structure and molecular interactions of *T. cruzi* EIF4E factors with their different partners (protein/RNA) in the protein synthesis machinery. RNA substrates will be synthesized and the structural studies of the initiation factors alone or in interaction with their partners will be performed by crystallography and/or NMR. Finally, they will identify the EIF4E homologs able to bind to cap-4 *in vivo* and they will characterize *T. cruzi* cap-4 binding complexes formed *in vivo*.

The results of this project will allow to understand the fundamentals of cap-4 and EIF4E function in protein synthesis of trypanosomatids, and to propose strategies for differential targeting of parasitic trypanosomatids.

Leishmaniasis, in its different forms, has been detected in over 100 countries, with 1 billion people at risk. 20,000-30,000 deaths due to visceral leishmaniasis annually.

## The study of the protective humoral immune response during Dengue virus infection in order to develop new vaccination strategies



#### Project PTR n° 212-2019

Scientific coordinator: Dr Tineke CANTAERT, Immunology Unit - Institut Pasteur in Cambodia Institut Pasteur International Network collaborators: Dr Giovanna BARBA-SPAETH, Dr Marie FLAMAND, Dr Pierre BRUHNS and Dr Milena HASAN (UTechS Cytometry and Biomarkers), Institut Pasteur (Paris) and Dr Philippe DUSSART, Institut Pasteur in Cambodia \*

#### WHAT IS DENGUE?

Dengue is caused by dengue virus (DENV), a virus which is transmitted by *Aedes* mosquitos. These mosquitos thrive well in populated urbanized areas, contributing to the spread of DENV. DENV belongs to the genus *Flavivirus* and comprises 4 distinct serotypes, DENV-1 to DENV-4, which co-circulate in the same endemic areas. Infection by one of the four serotypes results in a spectrum of clinical outcomes ranging from complete asymptomatic infection, to mild disease, classical dengue fever (DF), with or without hemorrhage, or dengue hemorrhagic fever (DHF) with plasma leakage leading to shock (dengue shock syndrome (DSS)) and other organ involvement.

Pathogenesis of severe dengue is complex. Most primary infections are mild and probably provide lifelong protection against the infecting serotype. In contrast, heterotypic secondary DENV infection (with a DENV serotype distinct from the primary infecting serotype) is the greatest risk factor for the development of severe disease requiring hospitalization. Currently, there is no treatment and there is one licensed vaccine available on the market, albeit has major drawbacks regarding both safety and efficacy.

#### **VACCINE AND IMMUNE RESPONSE**

The vaccine can only be administered to individuals who have been infected with DENV previously and is therefore not suitable for prevention of the disease in high endemic countries such as Cambodia. Vaccine development for the prevention of dengue infection is challenging because there are 4 different serotypes of dengue virus and an individual can become infected with different serotypes during life. This generates cross-reactive immunity where previous infection with one serotype leads to a mismatch between the immune response and the current infecting serotype, resulting in an exacerbated and uncontrolled immune response. This can give rise to clinical symptoms of ranging severity. The mechanistic understanding of this phenomenom and insight on how to avoid it and re-direct the immune response is essential for the development of effective vaccines.

Half of the world's population live in areas where the mosquito and the virus are circulating.

Infection by the virus causes 100 million of clinical dengue cases, including half a million hospitalizations and 22.000 deaths, mainly among children, each year.

Dengue is increasing at a higher rate than most other communicable diseases, with a doubling in incidence every 10 years since 1990

The current project brings together several scientists from Institut Pasteur International Network in the field of immunology and virology to study the immune responses initiated after dengue infection that provides protection from disease, but clears the virus efficiently. For that, the team will compare the generation of the humoral immune response after DENV infection in asymptomatic children to children with severe symptoms requiring hospitalization.

Recombinant viral proteins derived from clinical isolates will be used in order to increase the biologic relevance of the work. The researchers will investigate the phenotype of IgG secreting cells during DENV infection at high resolution by droplet microfluidics solutions. In addition, they will be able to investigate the B cell response during DENV infection by cutting edge single cell RNA sequencing technologies. Finally, the scientists will couple antigen-specificity to antibody effector functions by investigating antibody functionality with an array of established cell based assays.

The proposed work will provide ground-breaking insights into host protective mechanisms during dengue virus infection. The project aims to describe in detail the many aspects of the generation of a humoral response that confer protection after secondary dengue-virus infection. The results will establish a foundation for the development of new vaccination strategies and will identify novel correlates of protection to be used in vaccine efficacy studies.

Since 2012, dengue is the most important vector-borne disease of humans and likely more important than malaria globally in terms of morbidity and economic impact.

## Identify risk factors and routes of transmission of the monkeypox virus to prevent future infection



#### Project PTR n° 218-2019

Scientific coordinator: Dr Emmanuel NAKOUNE, Arbovirus, emerging viruses and zoonosis Unit - Institut Pasteur in Bangui

Institut Pasteur (Paris) collaborators : Dr Arnaud FONTANET, Dr Tamara GILES-VERNICK, Dr Jean-Claude MANUGUERRA and Dr Antoine GESSAIN \*

#### WHAT IS MONKEYPOX?

Monkeypox is a zoonosis caused by a virus member of the *Orthopoxvirus* genus (as smallpox virus) in the family *Poxviridae*. This disease has been reported primarily in forested areas of Central and West Africa and is occasionally exported to other regions.

There are two distinct genetic groups (clades) of monkeypox virus - Central African and West African. Human infections with the Central African monkeypox virus clade are typically more severe compared to those with the West African virus clade and have a higher mortality.

The virus is transmitted to humans from animals. It is suspected that some species of squirrels or Gambian rats may serve as the animal reservoir, but this needs to be confirmed. Monkeys, when infected, become sick and are not considered to be the reservoir. Until now, human-to-human transmission is relatively limited. The virus is transmitted by contact with blood, body fluids or cutaneous or mucosal lesions.

The clinical presentation of monkeypox resembles that of smallpox. The illness begins with fever, headache, muscle aches, swelling of the lymph nodes and progresses to the appearance of skin eruption (evolving from macules to vesicles and pustules). The rash tends to be more concentrated on the face and extremities than on the trunk with characteristic lesions of the palms of the hands and the soles of the feet.

There is currently no vaccine and no specific treatment.

#### THE CURRENT LIMITATIONS IN MONKEYPOX INFECTION UNDERSTANDING

Over the past two decades, there has been an apparent increase in the frequency of outbreaks, the size of each outbreak and in the secondary transmission associated with outbreaks, with the Democratic Republic of Congo and Central African Republic the most heavily affected countries. In recent years, the geographic extent of disease reporting has expanded into Nigeria and the savannah of South Sudan, and Cameroon. In addition, the reporting of human cases of monkeypox to non-endemic areas highlights the spread of monkeypox around the world and the threat of this virus to global health security in an increasingly interconnected world.

It also highlights the current limitations in infection prevention and control measures specific to monkeypox. While direct or respiratory contact with an infected human are known to result in secondary transmission, further risk factors and routes of transmission between humans remain unclear.

Moreover, there is neither a specific vaccine nor treatment for human infection. Smallpox immunity is understood to provide cross-immunity against monkeypox infection. However, smallpox was declared eradicated by the World Health Organization in 1980 and the subsequent discontinuation of smallpox vaccination activities is thought to result in an increasingly larger proportion of the population with no protection against Orthopoxvirus infection.

Understanding the animal reservoir and the types of exposures of human populations to the animal reservoir are critical for limiting zoonotic transmission and thus reducing further spread in the human population.

\* This project also involves / involved the participation of external partner(s).

The first case of human infection with monkeypox was described in 1970 in the Democratic Republic of the Congo.

In 2019, cases of monkeypox are still reported in the Central African Republic, the Republic of Congo, the Democratic Republic of Congo, Cameroon and Nigeria.

In 2019, monkeypox imported cases from Nigeria are reported In United Kingdom, Singapore and Israel.

Teams from Institut Pasteur of Bangui and of Paris as well as external partners will combine their complementary expertise (epidemiology, virology, anthropology, zoology and environmental ecology) to develop a One health comprehensive approach for studing monkeypox in Central African Republic. They will investigate on previous outbreaks of monkeypox using demographic, clinical and laboratory data as well as information on animal and human exposures to assess the risk to public health of monkeypox outbreaks. They will use a combination of anthropological approaches to provide a qualitative understanding of monkeypox among the affected communities. They will sequence and compare the viral strains for a better understanding of this virus and for the development of a field diagnostic test and a more specific serological assay for detection of monkeypox infection. They will try to identify the animal reservoir using an existing collection of animal samples stored in Bangui as well as extensive sampling of target wild animals. Finally, they will use both narrow and broad scope analyzes to assess the ecological characteristics of areas with documented monkeypox outbreaks, as well as the changes in these characteristics over time.

This project will strengthen capacity in Central African Republic for detecting and responding to outbreaks of monkeypox, reduce human-to-human transmission and to manage and control outbreaks of emerging zoonotic diseases in Central Africa, such as monkeypox. Furthermore, this is an approach that is applicable not only for monkeypox, but for other emerging zoonotic diseases.

Approximately 2-10% of all infections are fatal with most deaths occurring in younger age groups.

## Computational imaging approaches to investigate the role of mechanical forces in the colonic tissue to understand the microbiota colonization and the development of infectious enteric diseases



#### Project PTR n° 232-2019

Scientific coordinator: Dr Elisabeth LABRUYERE, Bioimage Analysis Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Hein Min TUN, Hong Kong University - Pasteur Research Pole, Dr Nathalie SAUVONNET, Institut Pasteur (Paris) \*

#### WHAT ARE MECHANICAL FORCES

Many in the biomedical sciences are beginning to recognize that cells are not only able to sense biochemical cues but also physical factors such as force, geometry and matrix elasticity, and that these can play critical roles in defining cell and tissue functions, morphology and regeneration as well as human physiology and pathology.

Mechanical forces trigger many cellular functions and change the geometry and physical properties of a tissue. This relationship can be exploited to indirectly characterize pathological (e.g.: tumor development and cancer cell metastasis) and physiological (e.g.: embryogenesis) changes or to study basic properties of cells and tissues. Presently biophysical properties of a tissue are measured with sophisticated and expensive instruments often in an invasive manner.

#### **RELATIONSHIP BETWEEN TISSUE MECHANISMS AND MICROBES**

Nevertheless, many progress have been made in the understanding of how mechanical inputs regulate cell behavior and tissue homeostasis. However, very few studies address these questions during the interaction of the tissue with microbes. For example, with the gut microbiome which has a central role in human health and disease, and with pathogens during infectious processes. Despite an increasing amount of knowledge of the role of the microbiome and on the molecular machineries evolved by pathogens to infect human cells, an important field of investigation remains on how they adapt to or modify the mechanical forces encountered in the host tissues, and what are the impacts on those tissues.

To answer these questions, tools are still lacking to quantify the cell mechanics within a tissue, in a spatiotemporal manner, without being invasive.

Amoebiasis is the third deadliest parasitic disease in the world.

Shigellosis kills hundreds of thousands of people around the world every year, especially children under the age of 5.

Mathematicians, biophysicians and biologists from 2 instituts of IPIN (Institut Pasteur Paris and Pasteur Research Pole Hong Kong) will combine their expertise to provide an integrated framework to analyze cell mechanics by combining imaging, and biophysical and computational approaches and to investigate the relation ship between host tissue mechanical forces and microbes. For that, they chose the colon as tissue model as it is in contact with the microbiome and exposed to pathogens, and is subject to important mechanical forces like peristaltic motion. First, they will develop a tool reproducing the features of the gut using organ-on-chip technology mimicking the composition, the tridimensional structure, and the peristaltic movement of the colonic tissue. Then, they will study the impact of mechanical streching on cellular shape and tissue architecture in the presence and absence of the microbes by incubating incubate the colon surrogate with commensal microbes and two enteric pathogens: the bacteria Shigella flexeneri and the amoeba parasite Entamoeba histolytica (which are major threats to public health worldwide causing dysentery and destruction of the colonic tissue). Finally, they will explore the role of the mechanical parameters in the microbiota colonization and in the pathogens invasive process by performing spatiotemporal analysis thanks to development of algorithms.

The tools obtained at the end of the project (the gut-on-chip colonised by microbiota and algorithms) will help at drug screening and testing and open new ways to study the mechanobiology of the interaction between microbiome, microbes and tissue.

Futures studies may reveal new drug targets (cellular and microbial) linked to tissue mechanics or may guide implementation of microbiota as therapy to improve gut health and to help at fighting some infectious and non-infectious human diseases. Lack of knowledge of the impact of mechanical stresses on tissue invasive process.

Need to develop non invasive methods to quantify physical parameters of tissue architecture.

## Identify new active compounds against multidrugresistant malaria parasites



#### Project PTR n° 233-2019

Scientific coordinator: Dr Ludovic HALBY, Epigenetic Chemical Biology Unit - Institut Pasteur (Paris) Institut Pasteur International Network collaborators: Dr Artur SCHERF and Dr Patricia BALDACCI (Center for the Production and Infection of Anopheles Platform), Institut Pasteur (Paris) and Dr Benoit WITKOWSKI, Institut Pasteur in Cambodia\*

#### WHAT IS MALARIA?

Malaria is a tropical disease due to a parasite named *Plasmodium*. There are five species of *Plasmodium* that can infect humans, the deadliest one being *P. falciparum*.

The parasite is transmitted to humans through the bite of an infected *Anopheles* mosquito during a blood meal. The parasite will rapidly reach the blood and stop in the liver where it will invade a cell and multiply inside the cell for approximately one week. This liver phase is silent and enables the parasite to grow from one parasite to more than 1.000. After one week, the parasite will make the cell burst and will be released in the blood again where it will invade red blood cells of the human host. This phase is called the blood stage and is responsible for the symptoms. Every 48h, each parasite will enter a new cycle in which, from one parasite, it will give approximately 30 new parasites that will invade new red blood cells. Symptoms are non-specific and look like a flu: fever, shivering, muscular pain, headache and nausea are the most common ones. If not treated in time, the disease can rapidly evolve in a life-threatening state, mainly characterized by a failure of multiple organs and coma.

Treatments are available to kill the parasite, but *Plasmodium* is becoming more and more resistant to these therapies.

#### DRUG-RESISTANT PARASITES AND NEW THERAPEUTIC STRATEGIES

*Plasmodium* is becoming more and more resistant to therapies, especially in South-East Asia where treatment efficacy is compromised. The threat is that the resistant parasites reach Africa, as it already happened by the past with the drug chloroquine, because the disease is deadlier there. There is thus an urgent need to develop new treatments that kills the drug-resistant parasites.

This pathogen uses phenotypic plasticity which means that it has the ability to change its behavior, morphology and physiology to adapt to changing environment. Epigenetic mechanisms play a major role in stage specific development and switch to different life cycle stages such as transmission stages. Epigenetic mechanisms are tightly regulated in time and are widespread in humans, animals, plants and pathogens. It has emerged that the modifications induced during epigenetics mechanisms play a major role in diseases particularly in infectious diseases such as malaria. These mechanisms can be targeted to kill Plamodium parasites and block parasites proliferation, thus avoiding the generation of new drug resistance.

It is a global health problem, still causing more than 450.000 deaths each year (mostly African children) and over 200 million infections.

Considering the lack of an efficient vaccine and the increasing threat of resistance in the parasite and vector, new drugs that target resistant parasites are of highest priority to combat malaria.

Four teams from two institutes of Institut Pasteur International Network (Paris and Cambodia) will combine their highly complementary skills (chemistry of epigenetic drugs, biology and epigenetics of the malaria parasite, *Plasmodium* transmission and *Plasmodium* drug resistance in the field) to develop alternative therapies by targeting epigenetics mechanisms. For that, they propose an interdisciplinary approach combining chemistry and biology to find new drugs to fight malaria.

In a recent collaborative study, two teams of the consortium have produced data revealing novel chemical structures that kill very efficiently drug resistant *Plasmodium falciparum* blood stages. Based on their preliminary data, they will identify new antimalarial drug candidates, then they will validate the effect of these new compounds in animal models and in the different life cycle stages of *Plasmodium* isolates from malaria patients. Finally, they will characterize the mode of action of these compounds.

The project should result finally in potent new drugs reaching clinical trials. In addition, the project will bring a deeper understanding of the epigenetic processes and its actors in malaria infection. This will open the road to new therapeutic strategies to answer to the urgent need of therapeutic alternatives in the taming of malaria.

## Development of field tests for rabies diagnostic to improve surveillance and control of this disease



#### Project PTR n° 237-2019

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#### WHAT IS RABIES?

Rabies is an infectious viral disease caused by rabies virus (RABV) (*Lyssavirus* genus) that attacks the central nervous system of the mammals, including humans. It is spread through a deep bite or scratch from an infected animal (domestic dogs and wild animals such as bats, foxes,...), usually via saliva. It affects mainly the poorest populations in rural regions of the low- or middle-income countries in Asia and Africa.

After the incubation period which can range from a few days to a few months, the first symptoms appear that are often nonspecific such as fever and headache. Once the virus progresses and cause inflammation of the brain, the symptoms can include slight or partial paralysis and various neuropsychiatric disorders such as anxiety, agitation, hallucinations and spastic spasms such as hydrophobia then they give way to coma and death (often by respiratory arrest) within a few hours or a few days.

After rabies exposure, the post exposure prophylaxis consists of extensive washing of the wound and application of the antiseptic and also a vaccination sometimes combined with serotherapy. The post exposure prophylaxis should be administered as soon as possible to prevent the onset of symptoms and death. Vaccination is an effective tool for the prevention of rabies, both in human and in animal.

#### DIAGNOSTIC

The diagnosis is essential, not only to have reliable data and a precise epidemiological picture of the disease, but also to implement as soon as possible the postexposure prophylaxis to the people exposed to the positive-diagnosed animal, as well as to identify and take in charge the relatives of the rabid patient which could have been in contact with this patient or/and with the animal source.

To date, the confirmation of suspected rabies human cases relies exclusively on laboratory analysis and it is not yet possible not detect the presence of the virus before the first clinical signs. The gold standard technique is the detection of viral antigens in brain biopsy. However, this technique is only possible at the post-mortem stage and it is rarely achievable in local settings, due to the difficulty to obtain such invasive tissue. Alternative samples and techniques have been developed for the *intra-vitam* diagnosis of human rabies, with the detection of viral nucleic acids in serial saliva samples and/or in skin biopsy by RT-PCR (real-time or conventional). Although these techniques are sensitive, they are expensive and can only be performed in centralized and well-equipped reference laboratories. Unfortunately, such laboratories remain sparse in low- or middle-income countries in Asia and Africa. Similarly, the diagnosis of animal rabies is also exclusively performed on brain biopsy at the *post-mortem* stage. However, this test is not reliable for non-invasive samples such as saliva, due to an overall lower sensitivity when analyzing biological specimens other than brain biopsies.

This viral disease is still responsible for an estimated 60,000 human deaths per year, due to dog-mediated rabies for 99% of cases.

40% of people bitten by suspect rabid animals are children under 15 years of age.

Scientists from 3 institutes of Institut Pasteur International network (Paris, Cambodia and Madagscar) with complementary expertise will bring together in order to to develop and to validate, both in laboratory conditions and in field settings, the first point of care tests (POCTs) for the rapid detection of the etiological agent of rabies (with rabies virus - RABV) in humans. In addition, these POCTs will also represent the first POCTs applied to the diagnosis of animal (dog) rabies on non-invasive samples. Ideally, they will meet all the criteria of such tests: affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and lastly delivered. The strategy will be based on the detection of the viral genome in saliva and will relies on the lateral flow device technology to interpret and validate the results with naked eyes. In the project, these POCTs will be first developed and validated in laboratory, before being evaluated in local settings in two rabies endemic countries with Madagascar and Cambodia, at the beside of the patient or at the footside of the dog, in field conditions. In addition, this implementation of this project in the two regions of interest will contribute to the reinforcement of the local network of the surveillance of both human and animal rabies.

At the end, this project will fulfil a major gap in the surveillance and the control of rabies, with the development of the first easy diagnostic tools dedicated to the confirmation of rabies in humans and animals on non-invasive samples. The use of such POCTs will allow to perform the confirmatory diagnosis even in delocalized and rural areas, thus contributing to the evaluation of the real burden of the disease as well as the on time public health and/or veterinary interventions requiring to manage the consequences of confirmed positive cases.

## Better knowledge of antibiotic sensitivity will pave the way to the development of new strategies to fight antibiotic resistance



#### Project PTR n° 245-2019

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#### **ANTIBIOTIC RESISTANCE**

Antibiotics are drugs used to fight bacterial infections. The discovery of these drugs has saved and continues to save many lives. However, their effectiveness is threatened by the ability of bacteria to adapt and resist treatment.

The misuse and overuse of antibiotics accelerate the antibiotic resistance development in bacteria. In addition, the presence at low concentations of antibiotics in the environment (wastewater plants, soil and water) leads the bacteria to develop strategies to survive and adapt, contributing to the appearance of new resistant bacterial strains. The bacteria can become resistant to antibiotics either through mutation or by acquiring a resistance gene that confers resistance to one or more antibiotics.

Antibiotic resistance has become a major public health concern that challenges the ability to treat bacterial infectious.

#### AMINOGLYCOSIDE CLASS OF ANTIBIOTICS

Aminoglycosides (AGs) are antibiocs known for their high efficiency against Gramnegative bacteria (such as *Pseudomonas, Acinetobacter* and *Enterobacter*) but their use is restricted to life threatening infections because of their nephrotoxicity and ototoxicity and optimized dosing strategies are determined to ally high efficacy with low toxicity. Elucidation of AG sensitization mechanisms in bacteria will allow to take advantage of them to use smaller effective doses of AGs and thus safely treat a wider proportion of infections.

Recently, the RavA-ViaA genes were identified in *E. coli* and *V. cholerae*, two gram negative bacterial models, where they affect the antibacterial action of AG class of antibiotics. However, the mechanism of this process remains elusive. In order to shed light into how the expression of these genes increases bacterial susceptibility to AGs, it's important to understand in which conditions these genes are expressed, which bacterial functions are necessary for sensitization, which bacterial processes are affected by these genes and which bacteria possess them.

Antibiotic resistance is a major public health, social and economic problem, and has been declared "the greatest and most urgent global risk" by United Nations in September 2016.

Antibiotic resistance can affect anyone, of any age, in any country.

The originality of this project resides in joining the expertise of three research groups of the Institut Pasteur (Paris), with a long standing tradition of studies on bacterial stress responses, antibiotic resistance, genomic plasticity and evolution, using various approaches.

In this project they will investigate the molecular mechanism by which the RavA and ViaA complex increases the susceptibility of Gram-negative bacteria to the AGs. The impact of RavA and ViaA on the different steps of AGs uptake will be investigated using genetics and physiological tests. A clue will likely be provided by identifying partners of RavA and ViaA. For this, high throughput genomic and genetic approaches will be used (transposon insertion sequencing, genetic screen). RavA and ViaA genes are present in more than a third of  $\gamma$  - proteobacteria genomes. Hence search for co-occurrence of genes with RavA-ViaA will be carried out using computational approaches. Besides providing candidates for partnerships, this survey may provide indications on the propensity of certain bacterial species to be sensitized to AGs. It will also identify genes and functions co-occurring in species carrying RavA-ViaA that may be good candidates for experimental testing as functional partners.

This project will be of fundamental and biomedical interest, because basic knowledge on AG sensitivity paves the way to the development of new strategies to fight antibiotic resistance, through the use of fewer antibiotics, increase of the usability of existing molecules, and decrease of the risk of emergence of antibiotic resistance.

25,000 deaths a year resulting from antibiotic resistance in Europe.

## Generate an inner ear organoid to facilitate the study of the inner ear development and research on therapies for Human inner ear pathologies



#### Project PTR n° 272-2019

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#### **INNER EAR COCHLEA**

The cochlea, the mammalian auditory organ located in the inner ear, constitutes the first step of sound processing. The cochlea is a structure like the shell of a snail, filled with a liquid called the endolymph and is made up of a spatially organized mosaic of sensory cells, also called hair cells due to the antenna-like structure they harbor for sound detection. In the cochlea, the sound waves are transformed into electrical impulses by hair cells, which transmit this information to the brain via the auditory nerve. The brain then translates the electrical impulses into known and understandable sounds. The cochlea can be affected by diseases of different etiologies : genetic, infectious, toxic, injurious, degenerative. Damage to the cochlea causes mild to severe hearing loss. Although interventions to prevent, identify and address hearing loss can bring great benefit to individuals, few such interventions are currently available.

#### **INNER EAR ORGANOIDS INTERESTS**

Inner ear organoids are self-organizing 3D tissue structures that can mimic certain auditory structures, including sensory cells and their associated neuronal cells. Inner ear organoids could provide a cost-effective solution to model human hearing loss. Since these organoids can be established from both mouse embryonic stem cells and human induced pluripotent stem cells, they have emerged as promising *in vitro* models for basic research in developmental biology, biomedical research and translational applications in the field of hearing. One short-term hope is that inner ear organoids could be used as a tool to make mouse inner ear gene therapy amenable to Humans by screening for effective gene transfer vectors. However, these organoids currently lack the high spatial organization that is characteristic of the cochlea, which prevents them from being used as *in vitro* models for auditory functions.

Hearing loss affects more than 460 million people worldwide.

It is estimated that by 2050 over 900 million people will have disabling hearing loss.

This interdisciplinary project, which involves the concerted effort of four teams from the Institut Pasteur of Paris, the Institut Pasteur of Korea and external partners, represents the first step toward the generation of cochlear organoids. The goal of this proposal is to generate inner ear organoids that can recapitulate *in vitro* the initial steps of the formation of the cochlear sensory epithelium. To this end, they will propose to generate inner ear organoids in a microenvironment that implements the graded activity of some molecular factors called morphogens, known from mouse genetics studies to be critical for establishing the spatial organization of the auditory organ. They will combine microfluidics technology with high throughput imaging and stem cell biology, in order to study the conditions under which inner ear organoids become spatially organized. Although the approach will be first developed on rapidly growing organoids from mouse embryonic stem cells, it will be directly applicable to human pluripotent stem cells with minor modifications to existing protocols developed by scientists of Indiana University.

The culture system proposed in this project should facilitate the study of human inner ear development and research on therapies for inner ear pathologies. Inner ear organoids are also a promising model in the context of regenerative studies of the mammalian auditory system, whose sensory cells have become specialized to the point of losing the ability to regenerate during evolution. Nowadays, unaddressed hearing loss poses an estimated annual global cost of € 680 billion.

## Relationship between stem cells and infectious diseases (ie influenza viral infection) to a better understand multi-organ systemic effects of pathogens in human



#### Project PTR n° 291-2020

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#### **INFLUENZA VIRAL INFECTION**

The flu is a contagious infectious respiratory disease caused by the virus influenza which cause seasonal epidemics. Most people recover within a week but influenza can cause severe illness or death especially in people at risk, such as pregnant women, elderly, young children, individuals with chronic diseases and health care workers. Decline of independence after hospitalization has been reported in the elderly, as well as in specialized residences. This poses a number of issues, including an ever-increasing cost on national health care budgets.

Several common flu-associated symptoms affect patients, among them, myalgia which refers to highly debilitating muscle pain, or more rarely, myositis, which refers to transient muscle inflammation. These secondary symptoms can generate more damaging lesions for at-risk populations such as young children, ederly and patients with chronic diseases. Influenza-associated myositis (IAM) is a complication mainly reported in children where symptoms include lower leg weakness, pain, and difficulties to stand. Children affected by neurologic and neuromuscular disorders are a vulnerable population with high-risk factors for influenza-related complications .

Many researches are focused on respiratory complications causing death, but limited studies address the causes and consequences of the associated symptoms that are experienced by millions of people and that can be debilitating.

## CONSEQUENCES OF INFLUENZA INFECTION ON SKELETAL MUSCLES AND STEM CELLS

Focus on infectious diseases has been traditionally on the infectious agent, the physiology of the tissues that are the target of the infectious agent, as well as the overall response of the organism. It is believed that muscle and joint pain are secondary symptoms resulting from viral infections, which generate high levels of inflammatory molecules in the blood circulation that can impact on peripheral nerve function. The immune response that occurs in influenza- infected lungs generates the release of many cytokines into the bloodstream, reaching distal organs and causing the secondary symptoms, including myalgia. Among the numerous cytokines released in the body, several of them are known to have biological effects on muscles and muscle stem (satellite) cells (MuSCs) during regeneration. MuSCs are the principal cell type assuring muscle growth and regeneration. They are quiescent in resting muscles and activate, proliferate and differentiate during regeneration. Although the precise composition of their niche remains to be defined, multiple cell types and molecules have been identified as regulators of MuSCs

Each year about 3-5 million individuals develop a severe flu, causing 290 000 to 600 000 deaths.

Children and elderly patients are at higher risk of skeletal muscleflu related complications.

properties. Endothelial cells, stromal interstitial cells including fibro-adipogenic progenitors (FAPs), pericytes, are found in proximity to MuSCs and crosstalk with them through various signaling molecules. In doing so, they contribute to maintain MuSC quiescence, regulate their proliferation following muscle injury, and are implicated in their self-renewal after regeneration is complete.

Research on the etiology of muscle dysfunction in case of influenza infection are limited and no studies have addressed the response of MuSCs or other cell types to influenza infection.

#### **THE PROJECT**

This project is the result of synergistic expertise within the Pasteur network (skeletal muscle research and stem cell biology, infectious diseases and sepsis). It aims to investigate in detail the mechanisms and the consequences of how muscle stem cells sense and respond to acute systemic pathogen-driven inflammation. Their strong preliminary data have paved the way for them to explore this area further and position this topic at the forefront of international research.

Using a mouse model of influenza infection, they aim to identify the signaling molecules and mechanisms acting on MuSCs remotely from the site of infection. They aim to characterize the biological responses adopted by MuSCs in the context of viral inflammation. To do so, they will establish transcriptomic signatures of the muscle cell populations in influenza infected mice, as well as their inflammatory signature. Results provided by these analyses will be further investigated by biochemistry or cell biology approaches to test their hypothesis of mechanisms of action. Moreover, they are interested to explore if their observations on muscle stem cells in influenza-mediated inflammation could be an indicator of a more general stem cell response to acute systemic inflammation in other contexts. Therefore, they will explore another distinct systemic infection models, sepsis and compare the 2 paradigms in mouse models, one related to viral response, and the other, a polybacterial infection. The characterisation of the molecular basis and functionality of this phenomenon will have a significant impact on human health by revealing a role of systemic inflammation during and after infection, in association with symptoms and in the context of stem cell function. The results obtained from our studies will provide and novel path for research areas investigating the response of stem cells to pathogens in other tissues and organs.

Children affected by neurologic and neuromuscular disorders are a vulnerable population with high-risk factors for influenzarelated complications.

## Better understand of the different routes of ESBL-PE acquisition in newborns in Madagascar in order to identify new strategies to control this acquisition



#### Project PTR n° 303-2020

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#### WHAT ARE ENTEROBACTERIACEAE?

The *Enterobacteriaceae* are Gram negative bacteria found everywhere in soil, in water, and especially in the intestines of humans and animals. *Enterobacteriaceae* are a very heterogeneous family in terms of their pathogenesis and ecology. These bacteria are in fact either parasitic (*Shigella*, *Yersinia pestis*) or commensal (*Escherichia coli*, *Proteus mirabilis sp*, *Klebsiella pneumoniae*).

*Enterobacteriaceae* more specifically *Escherichia coli* and *Klebsiella pneumoniae* are mainly responsible of neonatal infections in Low income countries (LICs).

These bacteria can produce some enzymes called "Extended Spectrum Beta-Lactamases", which can break down some families of antibiotics, preventing them from working. These extended-spectrum beta-lactamase-producing enterobacteriaceae (ESBL-PE) are resistant to most of the antibiotics commonly used in LICs (penicillins and especially cephalosporins (3rd-4th generation)) which render the treatment of these ESBL-PE infections more difficult to treat. Antibiotics called "carbapenems" remain one of the last resort antibiotics to treat ESBL-PE infections which are neither available nor affordable for population living in these settings.

#### **ESBL-PE ACQUISITION**

*Enterobacteriaceae* are known to colonize the digestive tract, which represents the first step to develop potential neonatal infections. The newborn can acquire ESBL-PE at birth and during the first weeks of life from several possible sources, e.g. mother, health-care workers, others relatives, food and environment (surfaces or objects). Data regarding the acquisition of ESBL-PE in neonates are scarce in LICs. The few studies that focus on ESBL-PE acquisition in neonates were conducted in neonatal-care units, which are a very specific context compared to the community. Indeed, neonates are often premature and are exposed to specific factors increasing the risk of ESBL-PE acquisition (e.g. invasive procedures, contact with contaminated equipment or medical staff, high antibiotic consumption).

A common belief is that the ESBL-PE transmission from an ESBL-PE positive mother to her child during labour or delivery, called mother-to-child transmission, plays a key role in the acquisition of ESBL-PE for newborns. According to the literature, the scientists from this project were the first to conduct a community study in LICs (Madagascar and Cambodia) on the acquisition of ESBL-PE in newborns. Their findings suggest that ESBL-PE colonization of mothers might not

Severe bacterial infections are a leading cause of death in children under 5 in low income countries (LICs) with neonates bearing the highest burden.

It has been estimated that almost 7 million cases of neonatal infections possibly occurred each year.

be a predominant source of infant colonization. However, ESBL-PE carriage in household members and others sources from the community (food, objects, and surfaces) were not documented in their study. Consequently, predominant routes of ESBL-PE acquisition in neonates are still undefined in the community in LICs.

#### **THE PROJECT**

This project will be carried out thanks to the association of five teams from two institutes of Institut Pasteur International Network (Paris and Madagascar) presenting complementary expertise (epidemiology, anthropology, microbiology, bioinformatics, biostatistics, modeling). The objectives of this project are to identify the different routes of ESBL-PE transmissions to newborns in the community and to suggest new strategies to control ESBL-PE acquisition in newborns. To do so, they will recruit 60 newborns at delivery in Moramanga (a semi-rural area of Madagascar). A stool sample of the mother and of the newborn (meconium) will be performed at delivery. Then, home visits will be planned during the first month of life to collect data on factors which might drive ESBL-PE acquisition and stool samples from the newborn, the mother and all the household members. Also, samples from environment in direct contact with the newborn (food, surfaces and objects) will be performed at each visit. They will analyze ESBL-PE with last generation DNA sequencing methods in order to characterize species, resistance genes, plasmids and clones of ESBL-PE. To analyze these data globally, they will develop novel sophisticated analytical approaches combining mathematical modeling and statistics.

As local environments and practices influence strongly the interactions of the infant with its environment, they will therefore conduct an anthropologic investigation to put the epidemiological, microbiological, genomic and modeling results into perspective in a more global context.

These results will complement in an integrative way the others approaches and should allow the identification of the most acceptable interventions for local population to prevent ESBL-PE acquisition in neonates by taking in account local practices and environment.

In Madagascar, one of the poorest countries in the world, the burden is huge: the frequency of neonatal infection is more than 15 times higher in Madagascar compared to the USA.

## Better understand the virulence mechanisms of pathogenic leptospires in order to fight against leptospirosis



#### Project PTR n° 310-2020

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#### WHAT ARE LEPTOPSIRES?

Pathogenic *leptospira* are bacteria responsible for the zoonotic disease leptospirosis, which has a worldwide distribution and affects poor people from developing countries, mostly under tropical areas.

Pathogenic leptospires colonize kidneys of reservoir hosts (mainly rodents) and are excreted through urine into the environment. Human are infected by contact with soil and water contaminated by infected animals' urine. *Leptospira* penetrate hosts and rapidly disseminate to target organs (including kidney, liver, lungs) through the bloodstream. *Leptospira* are not obligatory intracellular pathogen but they may transiently survive and replicate inside macrophages.

The manifestations of this infection range from flu-like symptoms to a severe lifethreatening form characterized by multiple organ hemorrhages and failures. These symptoms are not specific of leptospirosis and they render this disease often underdiagnosed.

Currently, leptospirosis is treated by an antibiotic treatment that is efficient only at the very early stage of the disease when bacteria are present in the blood, but when *Leptospira* colonize host organs, they become more resistant to antibiotics. The vaccine used for controlling leptospirosis has limited efficiency due to the high number of *Leptospira* serovars and serogroups (more than 300 serovars according to their surface antigens divided into 24 serogroups, distint variations within *Leptospira* species). There is therefore a need for more appropriate treatments and vaccine to fight leptospirosis.

#### VIRULENCE OR ADAPTATION TO THE HOST ENVIRONMENT

Leptospira virulence mechanisms or how they survive inside hosts remain largely unknown. One very important determinant of *Leptospira* virulence is their ability to adapt to the host environment. In particular, pathogenic *Leptospira* are equipped to resist deadly oxidants produced by the host innate immunity and tissues. Oxidative stress and disturbances in the normal redox state of bacteria can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA.

The consortium of this project aims to explore various mechanisms that pathogen *Leptospira* employ to adapt and withstand oxidative stress during an infection. They have identified two transcriptional Peroxide stress Regulators, PerR1 and PerR2, that control genes encoding defenses against oxidants. Their findings suggest that the interplay between the PerRs involve complementary and non-redundant mechanisms necessary for the defense against oxidants and for virulence in pathogenic *Leptospira*.

More than one million cases of leptospirosis are currently reported annually in the word, with 10% of mortality.

Global climate change and the worldwide expansion of urban slums favor the emergence of leptospirosis and will probably lead to escalation of its burden.

The scientists from the Institut Pasteur in Paris and Montevideo with complementary expertise allowing the use of multidisciplinary approaches (transcriptomics, proteomics, infectiology, cell biology and microbiology) will investigate the regulation of the adaptive response to oxidative stress by PerR1 and PerR2 in *Leptospira*. To do so, transcriptomic and proteomic studies will be performed on *Leptospira* exposed to peroxide to identify the defense and repair mechanisms that pathogenic *Leptospira* solicit to withstand and recover from an oxidative stress. They will also unravel the respective role of the two PerRs in regulating the adaptation to oxidative stress and investigate how they cooperate in *Leptospira* virulence and ability to escape the host innate immunity.

This study should lead to the identification of novel virulence associated factors that could constitute novel molecular therapeutic targets to fight leptospirosis. Obtaining *Leptospira* mutants with attenuated virulence will also participate in developing new vaccines strategies to control leptospirosis.

Noteworthy, the number of reported cases of leptospirosis is also increasing in Europe.

## Relation between bacteria and cancer: Targeting epigenetic deregulations (notably DNA methylation) upon bacterial infection (*H. pylori* and *E. coli*) as an innovative approach for novel cancer therapeutic strategies



#### Project PTR n° 332-2020

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#### **COLORECTAL AND GASTRIC CANCER**

Colorectal cancer (CRC) usually begins with the non-cancerous proliferation of mucosal epithelial cells. This growth gives polyps and can continue to grow gradually for 10-20 years before becoming cancerous. CRC cells that proliferate into the wall of the colon or rectum can penetrate blood or lymphatic vessels at later stages, producing metastasis to distant organs. Certain dietary and lifestyle choices can promote intestinal inflammation and modify the intestinal microflora to stimulate an immune response, both of which can facilitate polyp growth and conversion to cancer.

Gastric cancer (GC) is often either asymptomatic or it may cause only nonspecific symptoms in its early stages. It is the reason why it is often diagnosed at a late stage and associated with poor prognosis. By the time symptoms occur, the cancer has often reached an advanced stage and may have metastasized. Unfortunatly, GC is difficult to cure unless it is found at an early stage. GC is a multifactorial disease, where both environmental and genetic factors have an impact on its occurrence and development. Its most common cause is the infection by the bacterium *Helicobacter pylori*, which accounts for more than 80% of the cases. Certain types of *H. pylori* strains are more virulent than others. GC is a malignancy of high aggressiveness with heterogenous nature and constitutes a global health problem.

#### **RELATION BETWEEN BACTERIA AND CANCER**

Cancer has long been considered mainly as a genetically-triggered disease, however accumulating evidences support the involvement of infectious agents especially in organs with direct contact with microbial communities. It has been estimated that 20% of global cancer burden is linked to infectious agents. Up to now H. pylori, the major risk factor for gastric cancer, is the only bacterium recognized as a class I carcinogen by the WHO. However, growing evidences associate different bacteria with cancer, as E. coli strains for colon cancer. Most of these pathogens deregulate essential functions as the maintenance of genetic stability, while others impact on the efficiency of therapies. Despite a growing number of studies, many mechanistic aspects remain to be elucidated. This is even more crucial, as it is more and more suspected that the presence of a second (or more) infectious agent(s) may be a critical catalyst for infection-related cancer initiation and development. In parallel, in the last two decades, epigenetics, notably DNA methylation, has also been shown to participate to cancer onset and progression by leading to tumor suppressors silencing. Of note, the profile of DNA methylation serves as markers to define molecular subgroups of cancers. One emerging question today is the potential link between these two complex and dynamic components of the carcinogenesis cascade: bacterial infection and epigenetics. For the development of novel therapeutic strategies, the targeting of epigenetic deregulations upon bacterial infection is an innovative approach.

Colorectal cancer is a global threat with more than 1.8 million of new cases and more than 800,000 deaths worldwide each year.

Colorectal cancer is already the second leading cause of cancer death in the world, and its incidence is steadily rising in developing nations.

The Four teams from two institutes of Institut Pasteur International Network (Paris and Greece) will combine their highly complementary skills (genotoxicity and epigenetic changes, oncogenic cellular signaling, chemical drug design, mechanism of pathogenicity, host signaling pathways) to study the mechanisms responsible for infection-driven carcinogenesis. To this aim, they will make a parallel between the host response to two pathogens-involved in cancer: H. pylori and E. coli. They will cross-study the gene expression deregulation linked to DNA methylation in response to one or the other and explore co-infections, as happens in the microbiota. They will investigate the consequences of this co-infection in vitro and in vivo at the molecular and phenotypic level, focusing on components involved in tumorigenesis and genome stability. Moreover, inhibitors of DNA methylation will be tested to evaluate their ability to diminish the tumorigenic potential of the infections. Indeed, the impact of DNA methylation on the protumorigenic properties of H. pylori and E. coli infection and co-infection will be investigated by testing a series of new DNA methylation inhibitors synthesized by one of the partner using a specific in vitro assay. The driven-hypothesis is that the co-infection by H. pylori in the gastric mucosa and E. coli in the colon should exert a synergistic effect to promote the severity of either H. pylori or E. coli-induced pathogenicity.

The achievement of this project will lead not only to identify new critical mechanisms in the association between infection and cancer, but also explore the impact of the co-infection. It will also pave the way to innovative perspectives for anticancer therapies with the identification of potential drugs that inhibit the epigenetic changes induced upon infection in the host. Finally the outcome of this project is of paramount importance for the investigation of the role in carcinogenesis of the microbial niche variation and associated dysbiosis.

Gastric cancer is the fifth most common cancer with 1 million new cases/year and the third leading cause of cancer deaths worldwide, with a rate of median survivals less than 12 months for the advanced stage.

Gastric cancer occurs twice as often in males as in females and its incidence rate rises progressively with age.

## Develop a new method to validate *Plasmodium falciparum* protective antigens targeted by immune T cells in order to accelerate vaccine development



#### Project PTR n° 337-2020

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#### **CONTROL AND ELIMINATE MALARIA**

Malaria is still a major public health problem in many parts of the tropical world. Control efforts have significantly reduced incidence and deaths attributed to malaria over the past two decades. However, further reduction in malaria cases appears to have stalled in recent years in many parts of sub-Saharan Africa. Continued application of currently available tools is unlikely to achieve the goal of malaria elimination in such regions with high transmission. Moreover, the rise and spread of drug-resistant *Plasmodium falciparum (Pf)* strains threatens one of the key components of the current malaria control strategy. The availability of an effective vaccine against *Pf*, which is responsible for ~97% of malaria cases, could break this impasse and be a game changer in global efforts to control and eliminate *Pf* malaria.

An efficient *Pf* malaria vaccine is still lacking. The development of vaccines with protective efficacy of at least 75% against clinical malaria targeting at-risk groups, and ideally capable of reducing parasite transmission at populational level is expected by 2030. The leading malaria vaccine candidate, RTS,S/AS01, which is based on *Pf* circumsporozoite protein (CSP), demonstrated an efficacy of ~40% against clinical malaria incidence in African children and is currently being evaluated in an implementation study in 3 African countries. While RTS,S/AS01 has demonstrated the feasibility of developing a vaccine for *Pf* malaria, there is a clear need for the development of next generation malaria vaccines with significantly higher efficacy.

#### **ANTIGENS AND IMMUNE SYSTEM**

Recently, using a *Plasmodium berghei* (*Pb*) rodent malaria model, the scientists of the consortium have discovered 7 protective *Pb* antigens. The combination of these antigens with *Pb* CSP notably induced sterile protection in 80-100% of immunized mice, versus 0-30% using *Pb* CSP alone. Sterile protection was lost following depletion of CD8+ cells, indicating that cytotoxic CD8+ T cells are responsible for the high level of protection observed. The cytotoxic CD8+ T cells are main effectors of cell-mediated adaptative immune response. Through binding to their T cell receptor (TCR), cytotoxic T cells recognize their cognate antigen presented on the surface of a target cell by a class I MHC molecule. Successful recognition of an antigen then leads to killing of the target cell.

However, the lack of a robust and cost-effective method to validate the *Pf* orthologs of these protective *Pb* antigens hinders the transposition of these results to a human malaria model.

Despite all control efforts of the last decades, malaria is still the deadliest vector-borne disease causing ~230 millions of new clinical cases and killing >400,000 persons every year.

Plasmodium falciparum is responsible for ~97% of malaria cases being the most important human- infecting plasmodial species.

Four teams from two institutes of Institut Pasteur International Network (Paris and UPS- Sao Paulo) will combine their scientific expertise (on plasmodial preerythrocytic stages, lentiviral vectorology, on humanized mouse models and on system vaccinology) to develop a novel method to map epitopes and validate protective antigens presented on the surface of infected human cells.

This method is based on the use of (i) HLA-A2 humanized mice to produce *Pf*-antigen specific human CD8+ T cells following immunization with lentiviral particles, (ii) immunopeptidomics to map epitopes of individual *Pf* antigens, (iii) epitope-indexed single-cell sequencing to identify the T Cell Receptor (TCR) sequence of antigen specific CD8+ T cells, and (iv) TCR-specific fluorescent reporter cells to validate the presentation of the epitope of interest on the surface of primary human hepatocytes infected with *Pf*.

This project will allow to select *Pf* protective antigens necessary for the development of malaria vaccines with higher efficacy. This method could also be applied for other antigens of intracellular pathogens targeted by T cells and thus accelerate vaccine development.

# Host pathogens interactions in humans infected with zoonotic foamy viruses



#### Project PTR n° 353-2020

Scientific coordinator: Dr Florence BUSEYNE, Epidemiology and Physiology of Oncogenic Viruses Unit - Institut Pasteur (Paris)

**Institut Pasteur International Network collaborators:** Dr Richard NJOUOM, Pasteur Centre in Cameroon, Dr Nicolas BERTHET, Institut Pasteur of Shanghai - Chinese Academy of Science and Dr Marija BACKOVIC, Institut Pasteur (Paris)

#### WHAT ARE FOAMY VIRUSES?

Foamy viruses (FV) are the oldest retroviruses, described as largely nonpathogenic in their natural animal hosts. Simian foamy viruses (SFVs) belong to the Spumaretrovirinae subfamily of retroviruses. They can be transmitted to humans, where they establish persistent infection akin to the retroviruses that led to the emergence of two major human pathogens, human immunodeficiency virus type 1 (HIV-1) and human T lymphotropic virus type 1 (HTLV-1). Retroviruses have the particularity of integrating their DNA genome into the DNA of the host cell, so they will be persist throughout the life of infected people.

There is increasing evidence for ongoing simian-to-human transmission of SFVs in many parts of the world where humans have contact with nonhuman primates (NHPs).

SFV infections have been reported in humans exposed to NHPs in zoos, primate research centers and in people living in Central Africa, Asia and South-America, who are frequently in contact with NHPs. No case of inter-human transmission of SFV infection has yet been documented. Most of the infected humans have been injured by an NHP and are therefore the first human hosts of a zoonotic SFV strain.

#### SFV INFECTION AND HUMAN HEALTH

Human infection with SFV is unambiguously attributed to direct interspecies transmission. This situation is unique among retroviruses, making gorilla SFVs an ideal model for investigation of retrovirus transmission to humans, its consequences on human health, the mechanisms allowing viral persistence and those limiting inter-human viral spread. The scientists of this consortium recently performed the first medical analysis of SFV-infected hunters and described associations between chronic SFV infection, anemia and immune activation. But what are the consequences of SFV infection on human health ?

Envelope (Env) protein from SFV is the key surface protein that allows viral entry. The scientists showed that Env is the main target of the potent neutralizing antibodies in humans. They have identified individuals infected with two SFV variant strains, which can be differentiated by their Env protein. Therefore, it is important to investigate Env structure in order to understand the viral entry mechanisms and the functional features of the two Env variants that circulate in co-infected NHPs and humans.

Retroviruses are very common in the animal world.

Retroviruses crossed the barrier species on multiple occasions.

The four teams belong to three institutions of the Institut Pasteur International Network (Paris, Yaoundé and Shanghai) and have internationally recognized expertise in epidemiology and SFV immunology, in structural studies of viral glycoproteins, in medical virology and in the identification of pathogens. They will examine the consequences of SFV infection on human health and investigate the Env structure.

They will pursue the longitudinal medical follow-up of high risk Central African populations exposed to NHP, collect blood samples, to study the medical consequences of chronic SFV infection on human health. In addition, they propose an in depth characterization of SFV strains infecting humans.

They will also determine the Env 3D-structure using X-ray crystallography and cryo-EM. This structure will then guide the development of novel serological assays for coinfection diagnosis. Most importantly, the structure of Env will allow them to build functional studies on neutralizing antibodies that might be the key immune effectors preventing SFV spread in the human population.

This project will improve knowledge on the medical status of individuals infected with SFV, fundamental knowledge on the Env protein of this virus as well as the surveillance of SFV in high-risk populations.

Retroviruses can be the cause of various forms of cancer (leukemia, lymphomas, sarcomas, etc.), pulmonary and joint pathologies, immunodeficiencies (including AIDS in humans), and central nervous system degenerations. Sometimes they can also have no pathological consequences.

## Generation of forefront technologies to study microglial cells in the context of a devasting neurological disease (Alzheimer's disease)



#### Project PTR n° 377-2020

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**Institut Pasteur International Network collaborators:** Dr Dimitra THOMAIDOU, Hellenic Pasteur Institute (Athens, Greece), Dr Jean-Yves TINEVEZ, Institut Pasteur (Paris)

#### WHAT IS ALZHEIMER'S DISEASE?

Alzheimer's disease (AD) is the most common type of dementia, primarily affecting the aging population. At first, symptoms are mild, but they become more severe over time, making for patients harder to reason, remember recent events and recognize people they know. As a person's condition declines, they often withdraw from family and society. Although the speed of progression can vary, the typical life expectancy following diagnosis is three to nine years. AD is the only one in the top five causes of death that has no effective treatment or cure. Analyses of the brains of people with AD suggest that the presence of extracellular aggregates of amyloid- $\beta$  peptides (amyloid plaques) and intracellular inclusions of neurofibrillary tangles rich in microtubule-associated protein tau are pathological hallmarks of the disease.

#### ROLE OF MICROGLIAL ACTIVATION IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE

Recent evidence indicates an important role of inflammation and microglial activation to the development of amyloid plaques. Microglia is a population of glial cells located throughout the brain and spinal cord. As the main brain-resident immune cells, micloglia are central players in regulating key pathways in central nervous system inflammation. A number of genome wide association studies (GWAS) have linked AD triggering and/or progression to genetic variations in genes highly expressed by microglia. Several AD risk factors identified in GWAS are indeed expressed in microglia. Recent findings from the scientists of this project indicate that the second most important sporadic AD risk factor BIN1 exhibits high expression levels in human microglial cells. It is expressed by both neural and microglial cells. BIN1 products are membrane adaptor proteins that are implicated in cell membrane modelling dynamics and membrane-mediated endocytosis.

Functional studies to address the role of those genes in microglia are still in a quite preliminary stage. The contribution of BIN1 different expression levels in microglial cells in AD pathogenesis and amyloid plaques formation both in humans and animal models has not been investigated yet. Restoring or manipulating microglial function seems to be a promising therapeutic option for AD treatment, but to design effective therapies, modelling of microglia response both in human cell culture systems and animal models that resemble human conditions is required.

Worldwide, around 50 million people have dementia, and they are nearly 10 million new cases every year. Alzheimer's disease is the most common form of dementia and may contribute to 60-70% of cases.

Dementia is one of the major causes of disability and dependency among older people worldwide.

The project consortium is composed of 3 groups with distinct and complementary expertise in neural stem cell biology, neurobiology, induced pluripotent stem cells technology, animal models of neurodegeneration, *in vivo* imaging and digital image processing, all aiming to decode the contribution of BIN1 in microglia activation during AD progression. For that they will probe the consequences of changing BIN1 expression levels to the activation of microglial cells and clearance of amyloid- $\beta$  peptides/plaques. Using an *in vitro* model to study the interactions between human microglia and neural cells in brain organoids, as well as *in vivo* animal models, they will develop live-imaging approaches to observe in real time whether lower or higher BIN1 expression levels in microglial cells affect their ability of phagocytosis and amyloid plaque removal. They will also develop new technologies for the analysis of temporal series of 3D-images.

Through their approach, they will be able to quantify and compare the response of microglia to BIN1 in human and animal models, thus extracting significant information related to AD progression in humans. They anticipate that their results will contribute to understanding the role of BIN1 in AD pathology, shed light on the molecular mechanisms of neuroinflammation in Alzheimer's disease and help to lay the foundation for future studies on other aging-associated diseases. Therefore, successful realization of the project can also produce scientific results having a positive social impact in the near future. Alzheimer's disease prevalence is increasing as the population ages, which leads to the prediction of exponential growth in AD cases in the upcoming years from 850,000 in 2012 to 2,150,000 in 2040.

## The development of a safe and effective anticampylobacteriosis vaccine relying on chemical synthesis



#### Project PTR n° 380-2020

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#### WHAT IS CAMPYLOBACTERIOSIS?

Campylobacteriosis is a diarrheal disease caused by bacteria of the genus *Campylobacter*, most commonly *Campylobacter jejuni* (*C. jejuni*).

*Campylobacter* survives in a number of reservoirs, including contaminated water and domesticated animals (poultry, cattle, pigs). Poultry is the main animal reservoir of *C. jejuni* and the majority of infections in humans result from handling or consuming chicken meat and derived products.

After an incubation period of 2–5 days, common symptoms are mild to severe diarrhea, bloody diarrhea, stomach pain, nausea and/or vomiting, fever, headache and muscle pain. Usually, gastrointestinal symptoms are self-limiting but occasionally they will persist and result in hospitalisation. Antimicrobial therapy is seldom needed.

*C. jejuni* is the major trigger of the Guillain-Barré syndrome and the Miller Fisher syndrome, two auto-immune diseases that can severely and permanently impair the peripheral nervous system.

In addition to basic hygiene intervention, prevention of campylobacteriosis through vaccination is considered as a global public health priority. Yet, despite a diversity of attempts and candidates in clinical and preclinical development, there are no licensed *C. jejuni* vaccines, either for human or animal use.

#### **VACCINE DEVELOPMENT**

To be effective worldwide, any CPS-based vaccine against *C. jejuni* would have to contain the most common and prevalent serotypes. As a direct consequence of *C. jejuni* being the pathogen most frequently associated with Guillain-Barré syndrome, the use of any whole-cell vaccine approaches in humans is precluded due to obvious safety concerns. Alternatively, *C. jejuni* biosynthesize high molecular weight capsular polysaccharides (CPS) anchored at its outer membrane, which are crucial antigens for vaccine development. Functional *C. jejuni* CPS mimics obtained by chemical synthesis would represent an asset for the development of anti campylobacteriosis vaccines as the chemical approach would avoid not only risks of contamination coming from the required purification of CPS from bacterial cultures but also chemical fine-tuning toward enhanced immunogenicity. They would also serve as ideal molecular tools for the detailed investigation of the molecular features influencing the contribution of CPS to *C. jejuni* pathogenesis and as target of protective immunity.

*Campylobacter jejuni* infections are one of the leading causes of bacterial gastroenteritis worldwide, especially in children less than five years old living in developing countries.

*Campylobacter* is estimated to cause 400 million human cases of gastroenteritis per year.

*Campylobacter* infections are generally mild, but can be fatal among very young children, elderly, and immunosuppressed individuals.

Capitalizing on their strong background in the field of synthetic carbohydratebased antibacterial vaccines (*Shigella* and *Burkholderia*), scientists from the Centre Armand-Frappier (Canada) and Institut Pasteur (Paris) aim to develop a safe and effective vaccine against campylobacteriosis through chemical synthesis. To do so, they are going to 1) prepare a panel of oligosaccharide fragments related to the CPSs selected prevalent *C. jejuni* strains; 2) convert those into semi-synthetic glycoconjugates; 3) study the *in vivo* immunogenicity of the glycoconjugates in chickens; and 4) evaluate the reduced colonization of *C. jejuni* in cecal contents of chickens following immunization with the glycoconjugates.

Ultimately, this research project has the potential to become the first preclinical proof-of-concept of a synthetic glycan-based vaccine against campylobacteriosis.

Incidence and prevalence of campylobacteriosis have increased worldwide over the past decade and are most likely still underestimated.

## Refining the role of nicotinic acetylcholine receptors and their relevance as therapeutic targets in neuropsychiatric disorder endophenotypes



#### Project PTR n° 388-2020

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#### **NEUROPSYCHIATRIC DISORDERS**

Neuropsychiatric disorders, including neurodegenerative (e.g. Alzheimer's (AD) and Parkinson's (PD) diseases) and neurodevelopmental (e.g. autism, schizophrenia (SCZ) and attention- deficit/hyperactivity disorder) conditions are expected to have an increased impact on our populations. They are currently a major concern for global health and are extremely debilitating and costly diseases. Cognitive impairment, notably affecting memory, attention, executive functioning, visiospatial abilities and communication, is a shared core feature of several neuropsychiatric disorders.

For instance, SCZ is a dramatic burden for which there are no curative treatments and for which current medications have significant adverse effects. Autism spectrum disorders (ASD) is also one of the most prevalent neurodevelopmental disorders that affects around 1–2% of the population, and people with autism experience decreased life expectancy. Moreover, aging is associated with an increased number of neurodegenerative diseases, with AD being the most common neurodegenerative disorder affecting millions of people worldwide, followed by PD. These disorders are a humanistic burden, which not only concerns patients, but also caregivers, relatives, neighbors, and other individuals in a patient's daily life. They also represent a financial burden on society because of the potential for institutionalization and chronic use of treatments.

#### **IMPLICATIONS OF NICOTINIC ACETYLCHOLINE RECEPTOR**

There is a growing body of evidence linking alterations in nicotinic acetylcholine receptor (nAChR) number and/or function to neuropsychiatric conditions such as SCZ, AD, PD, and autism. The implications of nicotinic receptor modulation upon the clinical expression and progression of these disorders is currently under investigation, and these receptors represent promising targets for developing novel therapeutic drug development. Yet there is a huge need to propose novel preclinical models to better undestand how these receptors contribute to brain functions that are altered in the course of neuropsychiatric disorders.

The importance of developing novel psychoactive drugs for therapeutic medications in various neurodevelopmental and neurodegenerative conditions that are associated with complex behavioral impairments has been increasingly recognized. Although neuropsychiatric disorders were originally viewed as disorder-specific, it is now widely recognized that they can be shared by several conditions and that psychiatric disorders are heterogeneous. Using multiple endophenotypes (quantitative neurobehavioral traits that index genetic susceptibility for a psychiatric disorder) that cut across psychotic disorder diagnoses is likely to raise novel

Cognitive impairment is a shared core feature of several neuropsychiatric disorders such as schizophrenia and Alzheimer's disease and often implicates an altered acitivity of the prefrontal cortex.

Impairements in nicotinic acetylcholine receptor expression and function have been robustly associated with a number of neuropsychiatric disorders and these receptors may represent an important therapeutical target.

therapeutic strategies. In this context, determining novel key molecular players of several processes commonly altered across psychiatric diseases can provide rationale for developing novel drugs in the future, with more complete profile of action for patients. Demonstrating consequential impacts of specific nAChR subtypes on cognition-related endpoints and their underlying brain correlates, using preclinical models with high translational value, can provide important indications of the usefullness of a future pharmacotherapy based on allosteric modulators of these receptors.

#### **THE PROJECT**

Three teams from Insitut Pasteur (Paris) with strong complementary expertise propose to combine preclinical cutting-edge approaches (comprehensive cognitive testing, novel in vivo brain imaging, correlative analysis and modeling of human genetic variation of nAChRs) to refine the impact of the two nAChR subtypes mainly associated with psychiatric conditions in humans, in specific neurobehavioral processes implicated in several human conditions. They propose to study the role of nAChRs on transdiagnostic endophenotypes, in order to develop new insights into how impairments in nicotinic systems are shared across multiple neuropsychiatric disorders. First, they will use the touchscreen operant platform, a recently developed method for assessement of multiple cognitive processes in rodents which provides the possibility to address laboratory animals to a battery of complex behavioral tasks that are designed to assay multiple dimensions of cognitive function in humans, thereby showing high translational value. Second, they will implement the three-photon technology for brain imaging that enables deeper imaging than what was previoulsy done before with two-photon approaches, and will provide optimal conditions for measuring prefrontal cortex (PFC) activity in their rat models. Finally, they will examine the impact of nAChRs on social behavior at the group level, using a novel method for real-time tracking and automatized analysis of individual behavioral sequences simultaneously in several animals. This will allow them to study the impact of nAChRs on transdiagnostic endophenotypes, which represents a key to develop insights into how impairments in nicotinic systems are shared across multiple neuropsychiatric disorders.

Identifying the impacts of specific nicotinic receptor subtypes on cognitionrelated endpoints and their underlying brain correlates, using preclinical models with high translational value, can provide important indications of the usefullness of a pharmacotherapy based on allosteric modulators of these receptors. This project will provide novel insights in the relationship between several aspects of neuropsychiatric conditions. Implementing novel preclinical approaches will improve the understanding of the role of nicotinic acetylcholine receptors in neuropsychiatric disorders.
# Gain a better understanding of the strategies pathogenic bacteria employ to subvert host functions to replicate and cause disease and identify new therapeutic targets



## Project PTR n° 395-2020

**Scientific coordinator:** Dr Monica ROLANDO, Biology of Intracellular Bacteria Unit - Institut Pasteur (Paris) **Institut Pasteur International Network collaborators:** Dr Alejandro BUSCHIAZZO, Institut Pasteur in Montevideo, Dr Paola ARIMONDO, Institut Pasteur (Paris)

# LEGIONELLA PNEUMOPHILA

Legionella pneumophila is the bacterium responsible for legionellosis, a potentially fatal lung infection. The disease was first described in 1976, after 181 people died due to a severe pneumonia in Philadelphia, during an American Legion convention. *Legionella* are part of the aquatic flora and are found in many freshwater springs. They are heat resistant and can therefore be present in hot water tanks. In these aquatic environnements *Legionella* replicates intracellularly in protozoa, and thus it co-evolved with these eukaryotic hosts, mainly aquatic protozoa (e.g. amoeba).

Humans are infected by inhalation of contaminated aerosols. The bacteria present in aerosols are absorbed in the pulmonary alveoli and invade macrophages, cells of the immune system, wherein they replicate and destroy them.

After an incubation period of 2 to 10 days, legionellosis manifests as acute lung infections such as pneumonia, which can progress to 2 types of complications : irreversible respiratory failure and acute renal failure, which are then often fatal.

The bacteria, naturally resistant to the penicillins commonly used in the treatment of lung disease, can be effectively combatted with other antibiotics, like macrolides or quinolons if prescribed in time.

# STRATEGY USED BY BACTERIA TO CONTROL THE HOST CELLS

It is important to improve our knowledge of the biology of *Legionella*, their factors important for the pathogenesis, and their mechanisms of action, in order to design novel approaches for better diagnosis, surveillance and risk prediction.

After internalization by host cells, it is well established through studies on *L. pneumophila* that intracellular *Legionella* avoid host cell degradation and multiply in a distinct compartment named *Legionella* containing vacuole (LCV). Furthermore, the bacterium is able to secrete a large arsenal of effectors through a specialized type-4 secretion system. This allows *Legionella* to control the host cell response by injecting a high number of proteins with very diverse enzymatic activities. Interestingly, few of them have been identified as acting as nucleomodulins, proteins that modulate nuclear functions of the host during infection. Thus, such newly discovered nucleomodulins need to be characterized.

Legionella represent a considerable public health burden due to sporadic and epidemic outbreaks and nosocomial infections (mortality rates 5-20% and up to 50% for nosocomial infections), but they are also an economic problem due to the surveillance measures that must be taken to ensure that artificial water supply systems are not contaminated by Legionella.

The recent emergence of this disease is explained by its affinity for modern water supply systems such as cooling towers, air conditioners, jet baths, jacuzzi, hot water pipers, etc...

# **THE PROJECT**

Three teams from two institutes of Institut Pasteur International Network (Paris and Montevideo) with complementary expertise (*Legionella* infection models, protein crystallization and structure analyses and chemical approaches to decipher epigenetic modifications) propose to combine their different strengths to characterize one of those newly discovered nucleomodulins of *L. pneumophila* at the functional and structural levels in order to understand its role during infection. This nucleomodulin is predicted to encode for a histone lysine methyltransferase never described in a bacterial genome before. In this project they will analyse the activity and localization of this methyltransferase predicted to be injected by *L. pneumophila* into the host nucleus. They will also determine its crystal structure and will characterize its functional consequences on the host cell physiology, using both immunology based assays, transcriptome analysis and a chemical inhibitor library.

The project will generate fundamental knowledge on bacterial strategies to replicate inside host cells/cause disease. It could also allow the identification of new targets for therapeutics.

The incidence of the disease might be higher than recorded as not all cases are correctly diagnosed.



Exploring Host factors linked to phagosomal rupture by Mycobacterium tuberculosis using High Content phenotypic Screening and Lung-on-Chip techniques

Project PTR n°430-2021



## SCIENTIFIC COORDINATOR

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# PASTEUR NETWORK OR INSTITUT PASTEUR (PARIS) COLLABORATORS

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# **PROJECT SUMMARY**



# **KEYWORDS**

*Mycobacterium tuberculosis*, tuberculosis, TB, Mtb, multi-drug resistance, organ on a chip, lung, exvivo, anti-microbial resistance, siRNA screening, fluorescence resonance energy transfer, FRET, IPSC, LoC, host-pathogen interaction



# **TUBERCULOSIS AND HUMAN HEALTH**

**Tuberculosis (TB)** is an infectious disease that has plagued humanity for thousands of years and remains to date a global publiceven today. TB is caused by the bacterial pathogen *Mycobacterium tuberculosis* (Mtb) and usually presents as acute or chronic lung inflammatory disease, but may also manifest itself as extrapulmonary disease. While the widely used BCG vaccination does show benefits in infants and children, no satisfactory preventive measures against pulmonary TB in adults exist, which is the form of disease that drives the aerosolic spread of Mtb to new hosts, and thereby the epidemic. The currently available treatment includes at least 4 different drugs and needs to be taken for at least 6 months, which poses problems related to compliance and may lead to the emergence of drug-resistant infection.

This increasing number of multidrug-resistant strains constitutes a global health issue, and new therapeutic strategies are needed to reduce the treatment duration of drug-sensitive TB.

## MYCOBACTERIUM TUBERCULOSIS'S FATE IN HOST

There are still large knowledge gaps concerning the molecular mechanisms contributing to survival and persistence of *Mycobacterium tuberculosis* (Mtb) in the host lungs. A lot is still to learn on the Mtb infection parameters. To begin with, the Mtb is enclosed in the cell's phagosome and then disrupts this organelle to continue the infection process into the infected cell. Mechanisms of phagosome disruption and cytosolic survivance of the Mtb remains unknown to date, but, as many cells biological parameters may change depending on cytosolic access of a pathogen, the question of the intracellular fate of Mtb is crucial for our understanding of the infection.

Another striking lack of knowledge is regarding the invasion of Mtb at the tissue level. So far, the current methodologies (*in vivo* using small animals or 2D cell culture) failed to visualize the infection in a physiological context. The new "organ on a chip" technology allows biomimicking a human functional organ, enabling the study of the dynamics of host-pathogen interaction in a realistic environment.

### THE PROJECT

The project brings together three teams from Institut Pasteur Paris and Institut Pasteur Lille with complementary skills and will use most sophisticated and cutting-edge technologies to decipher the Mtb infection profile and pathways in human *ex vivo*.

The researchers will screen the host factors involved in phagosomal disruption using patient's derived induced pluripotent stem cells (iPSC). Very little is yet known regarding the invasion of Mtb at the tissue level. Indeed, lung tissues are a multicellular organization that is constantly exposed to mechanical forces like pressure, shear stress and other mechanical forces. The researchers will develop a lung-on-a-chip model from iPSC-derived human organoid to biomimic functional human organs.. This model coupled to quantitative imaging, will enable the study of the dynamics of host-pathogen interactions in a realistic environment. Applying this system to Mtb should shed light on the yet unanswered questions concerning the very early steps of mycobacterial invasion. The expected findings of the project thus will pave the way for novel and complementary intervention strategies, including host-directed therapy for countering antimicrobial resistance.

In 2020, an estimated 10 million people developed active TB, resulting in 1.5 million deaths and making it the second leading cause of death from an infectious disease.

There are around 500,000 multi-drug resistant (MDR) TB cases worldwide each year.



Tuning the ADAR1 RNA editing enzyme to boost type I IFN and NK cell innate immune responses in the model of HPVtransformed cells Project PTR n°432-2021

#### SCIENTIFIC COORDINATOR

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#### PASTEUR NETWORK COLLABORATORS

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# **PROJECT SUMMARY**



# **KEYWORDS**

Human papilloma viruses (HPVs), cancer, epithelial cells, immune response, ADAR, cell model, 3D bioprinting, live cell assay, cervical cancer, immune system, interferon (IFN)



# **HUMAN PAPILLOMA VIRUS**

Human papillomaviruses (HPVs) are double-stranded DNA viruses and are the most common sexually transmitted infectious agents worldwide. Although most HPV infections do not cause cancer, HPVs are associated with 5% of cancer development worldwide. In fact, some HPV types can cause cancer in the uterus, anus, penis, vagina, vulva, and oropharynx. To date, more than 180 HPV subtypes have been identified. Of these, 14 HPV are classified as carcinogenic, with the high-risk types (hrHPV) HPV16 and HPV18 responsible for near 70% of cervical cancer cases.

Persistence of HPV and passage through precancerous stages are required for the development of an HPV-associated tumor. It can take 10 to 20 years for a premalignant condition to develop into cancer because the virus is able to evade the host immune response and -among several immunoevasion mechanisms - to suppress type I interferon (IFN) signaling in infected cells. Vaccines exist, but insufficient availability and coverage make HPV infections a major global health problem. Therefore, it is important to identify novel mechanisms of hrHPV-mediated immune evasion to understand how this virus can consistently infect unreactive cells and promote cancer.

# ADAR1 IN THE IMMUNITY AGAINST CANCER

There are various pathways to activate immune responses. For instance, ADAR1 is considered as a "master regulator" of cytoplasmic innate immunity. Indeed, ADAR1 prevents immune system "false starts" against "own" RNAs. But, ADAR1 activity is elevated in most tumor types. Moreover, inactivation of ADAR1 in cancer cells has been shown to decrease their survival. That's why the question has been raised whether cancer cells use ADAR1 activity to evade immune surveillance. In addition, ADAR1 may also contribute to tumorigenesis through its role as a suppressor of IFN-I-mediated inflammation. These observations make ADAR1 an interesting candidate to be studied to better understand how hrHPVs bypass the immune system.

# **THE PROJECT**

By combining diverse expertise within the Pasteur network and using breakthrough technological approaches such as aptamer technology and 3D bioprinting of skin-like structures, the teams aim to identify novel mechanisms and regulators that suppress the innate immune response in hrHPV-transformed cells, with the ultimate goal of defining new therapeutic targets and interventions. The project will characterize, for the first time, edited RNAs and recurrent edited sites mediated by ADAR1, by RNA sequencing and computational analysis. ADAR1 interacting proteins will also be studied to identify novel partners. The project will provide new therapeutic modalities for the treatment of HPV-related cancers or for the development of HPV vaccines. In women, cervical cancer is the second most common cause of cancer death, with the virus detected in over 99% of cases.



# EvoLeish: Mapping mechanisms of evolutionary adaptation underlying fitness gain in New World L.infantum strains Project PTR n° 435-2021



#### SCIENTIFIC COORDINATOR

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#### **PASTEUR NETWORK COLLABORATORS**

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# **PROJECT SUMMARY**



#### **KEYWORDS**

Leishmaniasis, *L. infamtum*, New World *L. infantum*, American Visceral Leishmaniasis, adaptation, deletion, Molecular genetics, reverse genetics, forward genetics, genome editing, Epigenetics, functional genomics, parasite, innate immunity, host-pathogen



# VICERAL LEISHMANIASIS: A HUMAN HEALTH DANGER

Leishmaniasis is a disease caused by protozoan parasites of the genus *Leishmania*. Visceral leishmaniasis (VL), also known as kala-azar, is the most severe form and present high mortality rates without proper diagnosis and treatment. In the Americas the disease is a zoonosis caused by *L. infantum*, and clinical signs are characterised by irregular episodes of fever, significant weight loss, swelling of the spleen and liver, and severe anaemia. VL is a global public health problem, mainly affecting poor populations in Asia, East Africa, South America, and the Mediterranean region. The increase in lethality and the limited therapeutic options reveal the urgent need for therapeutic alternatives. Miltefosine is an oral drug available for leishmaniasis treatment, aproved to treat dogs (the main reservoir) but not for human VL treatment in Brazil. Currently, there are some effective leishmaniasis vaccines for dogs, but not for human.

#### FOCUS ON AMERICAN VICERAL LEISHMANIASIS HISTORY

American Visceral Leishmaniasis (AVL) is a persistent public health challenge in the continent and represents one of the major vulnerabilities in infectious disease management in Brazil. Although VL is a major national health problem, little attention has been paid to the key player: the New World (NW) *L. infantum* parasite itself.

Although they share the same origin, Old World and New World strains of *L. infantum* have been exposed to different environmental conditions over the past 500 years since the introduction of the parasites to the New World by the Spanish/Portuguese conquest, resulting in different evolutionary pathways for the two groups of parasites.

For instance, a significant evolutionary change has been described in L. infantum circulationg in the continent: the widespread distribution of an inherited genomic trait, a complete genomic deletion of 12 Kb, likely due to long-term adaptation to the ecology of the NW. It is likely that, coupled to genomic changes, there are biological traits that ultimately affect relevant epidemiological characteristics, such as drug resistance, virulence, and transmissibility by vectors.

# **THE PROJECT**

This project brings together three teams of Pasteur Network with complementary expertise in clinical epidemiology (team of M. Boité), molecular parasitology (team of G. Spaeth) and host/pathogen interaction (team of A. Descoteaux) to specifically evaluate differences in virulence and host immune response between NW *L. infantum* genotypes, and their association with the genomic 12Kb deletion cited above.

Recent data revealed the widespread distribution and high frequency of deletion carrying (DEL) *L. infantum* strains (126 of 177) and the occurrence of a divergent non-deletion (NonDEL) group in southwestern Brazil. Importantly, until now, this deletion has been detected only among NW strains, suggesting that this important genomic change is under positive selection in Brazil and thus highly relevant to the local dynamics of L. *infantum* infection. Through this multi-disciplinary project, the researchers aim at **1**) characterizing the phenotypes of Brazilian L. infantum isolates carrying the deletion (DEL) or not (NonDEL) by *in vivo* and *in vitro* infection models to assess immune response and virulence profiles, **2**) analyzing the functional-genetic and systems-level of their genomic and no-genomic adaptation, and **3**) assessing the immunomodulatory consequences of this adaptation.

This proposal will provide an unprecedented opportunity to uncover novel mechanisms of Leishmania environmental adaptation and fitness gain under clinically relevant conditions.

If not treated, the fatality rate of Viceral Leishmaniasis can reach 100% within 2 years.

VL is the second-largest parasitic killer in the world (after Malaria) responsible for an estimated 20,000 to 30,000 deaths each year worldwide.



# Spatially resolved proteomics and morphodynamics of hematopoeietic stem cell aortic precursors

Project PTR n° 439-2021



#### SCIENTIFIC COORDINATOR

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#### **PASTEUR NETWORK COLLABORATORS**

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### **PROJECT SUMMARY**



# **KEYWORDS**

Zebrafish, blood, blood cancer, stem cell, immunity, hematopoiesis, hematopoietic stem cell, HSC, embryogenesis, regenerative medicine, bona fide, transient niche, ArhGEF11, APEX2, zebrafish, in vivo, bone marrow



# THE ORIGIN OF HEMATOPOIETIC STEM CELLS

The body's immunity needs to constantly adapt to changing conditions with the support of blood cells that have the ability to self-renew. This renewal depends on a small number of hematopoietic stem cells (HSCs) inherited mostly from embryonic life. HSCs are mostly produced during embryonic development from major vessels. To produce HSCs with long-term replenishment potential, it is important to study their genesis in the developmental context, from arterial components, as well as their maintenance in adequate niches. Recent studies have successfully produced genetically engineered HSCs *in vitro*, which is encouraging for regenerative medicine. However, reprogramming *via* the expression of specific sets of transcription factors may lead to leukemia later on. Hence, studying - in native conditions -, the cellular and molecular events leading to HSC precursor cell biogenesis as well as the biological processes allowing robust acquisition/maintenance of their sternness potential is crucial.

### **HSC EMERGENCE REMAINS AN ENIGMA**

Live imaging in the zebrafish embryo model established that HSCs arise from hemogenic endothelial cells in blood vessels, a process known as the endothelial-to-hematopoietic transition (EHT). The consortium teams demonstrated that this non-conventional cell extrusion process occurs according to sequential steps requiring critical dynamic rearrangements involving adjoining endothelial cells. The teams are investigating how mechanical constraints impact on the morphodynamics of EHT cells, in particular blood flow. To date, however, a lot remains unknown regarding EHT biomechanics and regulation and how it affects their downstream cell fate. Further research is therefore needed to understand all mechanical and signaling components involved in hematopoietic stem cell genesis before achieving their production in vitro, for the regenerative medicine.

#### THE PROJECT

This research proposal aims to study the process of hematopoietic stem cell precursors emergence from the aortic wall, which is an unusual process of cell extrusion because exposed to the mechanical forces of blood flow. The research will be carried out by three specialized teams in cell/developmental biology, protein biochemistry/proteomics, and theoretical biophysics/image analysis. Two complementary approaches, dynamic 3D modeling based on high spatio-temporal confocal microscopy acquisitions and spatially resolved proteomics, will be used to develop the translational requirements of this research topic. The teams will work to characterize the essential biomechanical parameters of the emergence process, including the modeling of forces, intercellular rearrangements, evolution through time of junctional interfaces. They will also develop, in the zebrafish embryo model, proximity biotinylation using the APEX2 peroxydase so as to identify protein complexes involving ArhGEF11, a Rho-GTPase coupled to G-protein coupled receptor signaling and controlling junction recycling and actomyosin contraction. Ultimately, this research will contribute to unveil the mechanisms underlying this unusual process of cell extrusion that leads to the genesis of essential cell types that constitute our blood and immune systems.

HSCs emerge during a very narrow time-window of approximately 2 days during the embryonic development of vertebrate species



# Proof of concept study: targeting DENV NS1 to reduce viremia and alleviate plasma leakage

Project PTR nº 448-2021



#### SCIENTIFIC COORDINATOR

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# **PROJECT SUMMARY**



#### **KEYWORDS**

Virus, arbovirus, flavivirus, Dengue, DENV, NS1, high-throughput, high-content, screening, chemical library, BRET, infection assay, ELISA, preclinical, mouse model, antivirals.



# FLAVIVIRUSES COMPLEX HANDLE

Dengue (DENV), Zika (ZIKV), yellow fever (5FV), Japanese encephalitis (JEV), West Nile virus (WNV), and tick-borne encephalitis (TBEV) are transmitted by arthropods and can cause a variety of symptoms in humans, from a nonspecific febrile illness to more severe conditions such as encephalitis, meningitis, or hemorrhage and shock. Diagnosis of these diseases can be difficult because of overlapping early clinical symptoms and cross-reactive antibodies. In the specific case of dengue fever, despite preventive measures such as mosquito vector control and vaccine development, the number of cases reported worldwide has increased dramatically in recent decades, highlighting the need for specific therapy. Although drug discovery programs have focused on the viral and host targets required for infection, drug candidates tested in clinical trials have not successfully alleviated the condition in patients.

# NS1 VIRAL SEQUENCE AS A TARGET TO FIGHT FLAVIVIRUSES

Flaviviruses have RNA as genetic material that encodes proteins important for replication and immune response, including NS1. Recent research has shown that NS1 plays a key role in the virulence of flaviviruses such as dengue virus and is a potential target for antiviral drugs. Several studies have shown that deletion or mutation of the NS1 sequence can prevent viral replication. In addition, immunization or infusion of NS1-specific antibodies may provide protection against subsequent infection. These reports support the hypothesis that inhibition of NS1 biogenesis will reduce viral replication, immune escape of infected cells, and NS1-related pathologies.

# THE PROJECT

The project aims to (a) identify small molecules capable of inhibiting DENV NS1 biosynthesis either directly or indirectly, (b) identify those that are noncytotoxic, (c) reduce dengue infection *in vitro*, and (d) reduce virus progeny and NS1 secretion. Following a preliminary structure-activity relationship study, the safest and most effective molecules will be tested *in vivo* in an appropriate flavivirus infection model. The teams hope to provide proof of concept demonstrating that targeting DENV NS1 could be a good therapeutic option to combat dengue and related flaviviral diseases. By using high-throughput small molecule screening, *in silico* medicinal chemistry, and small animal models that recapitulate dengue disease characteristics, the consortium aims to develop a potential drug that can alter NS1 biogenesis and have potent antiviral activity. Between 2015 and 2019, number of deaths due to Dengue ranged from 8,573 to 22,169 per year.

Currently, 3 vaccines against Dengue exist, but no specific treatment is available.



High-Resolution virome of mosquitoes coupled to the quest for novel human arboviruses in Madagascar Project PTR n°458-2021



#### **SCIENTIFIC COORDINATOR**

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### PASTEUR NETWORK COLLABORATORS

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# **PROJECT SUMMARY**



# **KEYWORDS**

Arbovirus, mosquitoes, arthropod, emergence, outbreak, epidemic, diagnostic, screening, LIP, seroprevalence, NGS, viriome, Madagascar,



# **ARBOVIRUSES OUTBREAKS: MAJOR HUMAN HEALTH RISKS**

Arboviruses are viruses transmitted by arthropods such as mosquitoes that have significant social and economic consequences, including fatalities. Over the past 25 years, these viruses have caused dramatic outbreaks resulting in widespread morbidity and mortality. According to the World Health Organization (WHO), mosquito-borne diseases such as dengue, Zika, and chikungunya affect millions of people worldwide. In addition to the human cost, these outbreaks have significant economic consequences, particularly in low- and middle-income countries with inadequate health infrastructure. For example, the Zika virus, once considered a mild disease, has been linked to severe birth defects, including microcephaly, since 2015. Overall, arboviruses continue to pose a significant public health threat, and the burden of these diseases is likely to increase in the coming years. Effective surveillance, control measures, and vaccine development are needed to mitigate these risks.

### MADAGASCAR: A MICROCOSM FAVORABLE FOR RESEARCH

In addition to known viruses, other unknown or unexpected mosquito-borne arboviruses can infect a significant proportion of the human population in Asia or Africa without being detected because of asymptomatic, paucisymptomatic, or obvious but unrecognized viral infections. Islands are known hotspots for emerging mosquito-borne viruses because of specific factors that make them susceptible to epidemics. In the recent past, they have acted as amplifier hotspots for mainland regions. Madagascar represents a microcosm for globally relevant zoonotic disease research. The high risk of disease is overlaid by a range of socioeconomic and environmental circumstances that can influence the spread of disease, such as high population growth, rapid deforestation, climate warming, and changing rainfall patterns.

## THE PROJECT

The project combines high-throughput techniques for inventorying viruses in mosquitoes and then detecting human infections at well-defined humanmosquito interfaces in Madagascar. Initially, teams will collect mosquitoes in a selected area of Madagascar and analyze arthropod samples to identify novel human arboviruses and understand the typology of viruses that do not infect humans. The project will use a variety of techniques to identify and confirm the presence of new viruses, including phylogenetic analysis, molecular biology techniques, and immunoprecipitation assays. The project also includes capacity building and on-site training in pathogen identification/discovery using Next Generation Sequencing (NGS) technology. The long-term goal of the project is to disseminate similar programs and build capacity throughout the Institut Pasteur network to expand targeted diagnostics for arbovirus-like syndromes and elucidate the causes of viral emergence. Mosquito-borne diseases claim around 50,000 deaths yearly.

In Madagascar there are more than 200 species of mosquitoes referenced



# Tackling a Global Emerging Threat: Multidrug-Resistant Yeast Candida auris

Project PTR n° 459-2021



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# **PROJECT SUMMARY**



# **KEYWORDS**

Candida, Candidiasis, Candida auris, multi-drug resistance, fungi, antifungal drug, screening, highthroughput drug screening, HTS, kinase, mouse model, doxycycline, inducible expression, genetic ressource



# CANDIDIASIS BURDEN ON HUMAN HEALTH

Candidiasis is a fungal infection caused by the Candida species and can significantly impact human health. The severity of the infection can range from mild superficial infections such as thrush, affecting the mouth or genitals, to life-threatening systemic infections in immunocompromised individuals. The overgrowth of Candida can also lead to various chronic health conditions such as irritable bowel syndrome, chronic fatigue syndrome, and autoimmune diseases. Candidiasis can be particularly problematic in individuals with weakened immune systems, such as those with HIV/AIDS, cancer patients, and those taking immunosuppressive drugs. Additionally, the overuse of antibiotics and other medications, as well as lifestyle factors such as high sugar diets and stress, can contribute to the overgrowth of Candida and increase the risk of candidiasis.

# **CANDIDA AURIS SPECIFIC CASE**

*Candida auris* (C.auris) is an emerging drug-resistant pathogen causing recent hospital outbreaks worldwide and is highly transmissible. Little is known about the mechanisms underlying the pathogenicity and drug-resistance potential of C.auris. The emergence of multidrug-resistant yeast Candida auris has led to major hospital outbreaks worldwide, and conventional identification methods have limited accuracy. Therefore, there is a need to design strategies that use genetic and functional genomics approaches to investigate C. auris pathogenicity and drug-resistance mechanisms. In response to this need, the Pasteur Network has launched an initiative to create a genetic resource to study C. auris "decision-making" proteins, called kinases, and identify essential ones.

# **THE PROJECT**

The proposed project has three objectives. The first objective is to create a genetic resource in *C. auris* for functional studies of its pathogenicity and drug resistance traits, focusing on key decision-making proteins called Kinases. The second objective is to implement *in vitro* and *in vivo* functional analyses to identify essential kinases in *C. auris*. The teams will use phenotypic profiling and animal models of C. auris infection to identify central kinases that control drug resistance and virulence. Finally, the consortium will use chemical/drug-screening assays to target C. auris essential kinases, thus setting the ground for a scientific campaign against this global emerging threat. The project will be implemented in three work packages and will involve four members of the Pasteur Network directed toward fighting against C. auris. Overall, this project will provide valuable insights into C. auris pathogenicity and drug resistance, as well as lead to the development of novel antifungal drugs.

Candida infections are accompanied by high mortality rates that range up to 60%

C. auris has been identified in more than 30 countries.



# Centrosome and basal body function in human parasites

### Project PTR n°474-2021



#### SCIENTIFIC COORDINATOR

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# **PROJECT SUMMARY**



# **KEYWORDS**

Parasite, trypanosomatids, apicomplexa, *Trypanosoma brucei*, *Toxoplasmosa gondii*, flagella, centriole, centrosome, basal body, phylogeny, cell division, mass spectrometry, proteomics, cell model, fusion-proteins, RNAi, Immunofluorescence, protein structure



# HUMAN PARASITES: APICOMPLEXA AND TRYPANOSOMA

Parasitic infections caused by protozoan parasites have a considerable impact on human and animal health. Parasites **can be transmitted to humans by another host or, for some, during pregnancy**, where the parasite can be transmitted to the fetus.

The phylum **Apicomplexa**, includes over five thousand species of singlecelled parasites, all of which are pathogenic to humans and other vertebrates. Among the Apicomplexa are **Toxoplasma gondii (T. gondii)**, which causes toxoplasmosis, and species of plasmodium responsible malaria, one of the deadliest parasitic disease. **Trypanosomatids** are extracellular parasites responsible for notorius human and animal pathogies such as sleeping sickness caused by **Trypanosoma brucei (T. brucei)**. This disease can be fatal because the parasite invades the nervous system.

Although human infection with these parasites is declining, their socioeconomic impact remains a major burden on human health. In addition, while drugs exist for some of these parasites, **drug resistance occurs**, or drugs are not active against all of their life forms. There is a need to find new therapeutic approaches for these pathogens.

# FOCUS ON THE TOPIC

Despite their differences, Trypanosomatids and Apicomplexa share common features. For instance, the centriole, which is an essential cellular structure for those parasites as it allows the cell division (Apicomplexan) and the flagellar organisation (Trypanozomatids in general). More informations on these structures components would pave the way to new therapeutical targets and routes which could be exploited in the development of novel therapeutics from both Apicomplexa and Trypanosomatids. **Toxoplasma gondii** and **Trypanosome brucei** are amenable biological models to explore the basic biology of apicomplexans and trypanosomatids, respectively, and the evolutionary conservation of shared mechanisms of parasitism.

# **THE PROJECT**

This project follow up the ACIP grant (076-2017) which allows the teams to gain robust expertises and knowledges on both Trypanosoma and Toxoplasma. They already identified novel component of the Apicomplexan centrosome and their homologs in Trypanosomatids. Now, the teams aims to go deeper in the description of the different functional domains and roles of the newly identified centrosomal proteins of Apicomplexan and to compare it to their homologs in the Trypanosomatids. The project will bring valuable evolutionary perspectives which can be used for rational design of new drugs targeting essential parasites cellular structures.

About 30% of global human population is infected by Toxoplasma gondii.

55 millions of persons are exposed to the Trypanosome brucei



# Chemical RNA modifications in RIG-I-Like receptor Signaling

Project PTR n°483 - 2021



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# **PROJECT SUMMARY**



# **KEYWORDS**

Influenza A viruses, IAV, RNA viruses, pandemic, influenza, zoonic infection, Pattern recognition receptors, PRR, RIG -I-like receptor, RLR, RIG -I, MDA5, LGP2, synthetic RNA, RNA, BRET, NGS,



# THE INFLUENZA VIRUSES UNPREDICTABILITY

Influenza viruses are single-stranded negative sense RNA viruses that belong to the *Orthomyxoviridae* family and pose a serious threat to human and animal health. Influenza A and B viruses, such as H1N1pdm09, H3N2, B-Victoria, and B-Yamagata, are responsible for seasonal epidemics. Animal influenza viruses can occasionally be transmitted to humans, resulting in zoonotic infections and, less commonly, human-to-human transmission that can cause a pandemic. For example, the 1918 "Spanish flu" pandemic was the worst in history, claiming up to 50 million lives worldwide. Other major pandemics were caused by A(H2N2) in 1957, A(H3N2) in 1968, and A(H1N1)pdm09 in 2009. The current pandemic COVID -19 highlights that vaccine development can take time, which is hardly compatible with the crisis periods of pandemics. Therefore, broad-spectrum antiviral drugs that modulate the innate immune response should be considered as a means of inhibiting emerging RNA viruses, including new influenza viruses.

### **RLR RECEPTORS AS PROMISING THERAPEUTICAL CANDIDATES**

Pattern recognition receptors (PRRs) are important for the recognition of viruses and immune response to it in the body. They include the RIG -I-like receptor (RLR) family, which contains three important members: RIG -I, MDA5, and LGP2. Upon recognition of viral material, RIG -I and MDA5 trigger a cascade of events leading to activation of the innate immune response. LGP2 alone is not capable of triggering an immune response. LGP2 is an accessory factor that inhibits RIG -I-mediated signaling and, in synergy with MDA5, activates the immune response. RLRs are therefore an effective and attractive candidates for antiviral therapy, vaccine development, and cancer treatment. For this reason, synthetic RNAs have been developed as promising RLR modulators, but the exact processes behind the abilities of natural viral RNAs to interact with RLRs remain unclear. There is a great need to better understand them in order to develop new relevant therapeutic tools.

# THE PROJECT

This project aims to gain a better understanding of the post-transcriptional modifications of RLR RNA ligands that play a role in the innate immune response to viral infections. Specifically, researchers will use influenza A virus as a model. They'll take the RNA ligands specific to RLR and sequence them. Then, they will synthesize modified versions of those RNAs and develop an approach to detect RLR/RNA interactions *in vitro* and in living cells. Finally, reserchers will perform assays to identify specific RNA ligands that stop a broad spectrum of viral infections. This research brings together diverse disciplines such as virology, immunology, molecular biology, biochemistry, chemistry, and technological developments to gain insight into the structure and biochemical composition of natural RLR RNA ligands and develop new immunomodulators that can be used to improve human health and prepare for the next pandemics.

Since October 2022, the Centers for Disease Control and Prevention (CDC) has genetically characterized 1,693 influenza viruses.

A novel influenza virus strain emerges each year



# **Cyanobactins Against Microbes**

# Project PTR n°488-2021



#### SCIENTIFIC COORDINATOR

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# PASTEUR NETWORK COLLABORATORS

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### **PROJECT SUMMARY**



#### **KEYWORDS**

Cyanobacteria, cyanobactins, antibiotic-resistance, NGS, genome mining, antimicrobial, library platform, Syn-Cyanobactin



# **BACTERIAS AS DRUGS PRODUCERS**

About half of the drugs currently in use are derivatives of natural products synthesized by microbes. These include cyanobacteria, bacteria that perform photosynthesis and contribute to the balance of CO2 and O2 in the atmosphere. They are also adapted to a wide range of environmental conditions. This adaptability is partly due to the fact that these bacteria have a variety of proteins and production pathways, particularly antimicrobial toxins that favor their colonization of the environment. So, cyanobacteria have traditionally been used as source materials for pharmaceutical studies. These bacteria are the most important primary producers of cyanobactins on Earth, peptides with remarkable biological activities that show great potential as antimicrobial, anticancer, and antiviral agents. As antimicrobial resistance is a growing burden to human health, there is an urgent need to develop pharmaceutically relevant new compounds, and cyanobacteria are an interesting natural source for such development.

# CYANOBACTINS AS NOVELSOURCE OF DRUGS

Cyanobacterial biosynthesis provides ideal pathways for generating diversity and whose modularity can become an important source of natural products of societal interest asealy reproducible *in vitro*. This project consortium already has experience with this topic and has conducted a pilot study using genomes of cyanobacteria from Institut Pasteur collections. One of the conclusions is that the currently known cyanobacterial processes provide only a small glimpse of the potential of cyanobacteria to generate proteins of pharmaceutical interest diversity. A more comprehensive multidisciplinary approach is needed to unlock their full therapeutic potential.

\*Genomics to discover biosynthetic pathways of natural products and their potential interactions.

#### THE PROJECT

The Cyanobactins Against Microbes (CyAM) project combines synthetic biology, chemistry, structural analysis, and bioactivity assays to uncover the diversity, structure, biosynthesis, and activity of cyanobactins. In addition, the team will identify new cyanobacterins of pharmaceutical interest and gain a comprehensive overview of how they are produced in cyanobacteria. Then, the team will mimic production pathways *in vitro* to more efficiently generate cyanobactins of pharmaceutical interest. The production of pure cyanobactins identified in this project will provide the basis for testing their antimicrobial properties as a source of next-generation antibiotics.

There would be between 3000 and 8000 different cyanobacteria species (still on debate)

The teams already identified 79 novel cyanobactins precursors in the Microcystis and Planktothrix cyanobacteria strains



# Omic-based prognostic biomarkers of cervical cancer progression

### Project PTR n°492-2021



#### SCIENTIFIC COORDINATOR

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#### **PROJECT SUMMARY**



#### **KEYWORDS**

Cervical cancer, cancer, human papillomavirus, HPV, omics, bisulfite sequencing, sequencing, next generation sequencing, diagnostic, human cohort, methylation, genome, virus, Next Generation Sequencing, NGS



# **CERVICAL CANCER**

**Cervical cancer** is a serious health issue that affects millions of people worldwide, particularly women. According to the World Health Organization (WHO), **cervical cancer is the fourth most common cancer in women** globally, with an estimated 266,000 death reported in 2021. The impact of cervical cancer on human health is significant, as it can cause a range of symptoms such as vaginal bleeding, and pelvic pain, which can affect a person's physical and emotional well-being. In addition, cervical cancer can lead to long-term health problems, such as infertility. Proper vaccination against the pathogen Human Papilloma Virus (HPV) and **early detection strategies can significantly reduce the impact of cervical cancer**.

# STRATEGIES OF HPV DETECTION: NEED FOR OPTIMIZATION

Of the more than 200 HPV types that have been described and classified, only 13 genotypes have been associated with the risk of developing cervical cancer. Vaccination and screening are two approaches to preventing cervical cancer. Vaccination campaigns benefit younger generations of women and future generations, but it will take several decades for the health effects to become a reality. Screening programs can reduce the burden of cervical cancer faster and benefit current and future generations of women. Many molecular tests have been developed to detect viral DNA, but they do not distinguish between transitory and persistent HPV infections. Tests based on the detection of specific viral genes overexpression can distinguish between transitory and persistent HPV infections. Patients' cellular proteins can be used as biomarkers, to more specifically screen for lesions that may progress to malignancy, as a complement to screening based on molecular tests for HPV DNA detection.

#### THE PROJECT

This project was initially submitted in the framework of a collaboration between Institut Pasteur in Paris and two institutes located in Cameroon and China. In preparation for this project, the chinese team has developed **rapid diagnostic tools for the main HPV genotypes** as well as different methodologies to study both virological and cellular biomarkers, including HPV genome methylation and insertion into host genome. These different approaches have been validated on the Chinese female population in collaboration with the local gynecology department. Now, all these tools will be applied to identify female populations in Cameroon and women at high risk of developing cervical cancer. About 80% of people who have not had the HPV vaccine before they become sexually active will become infected with at least one type of HPV

The HPV genotypes number 16 and 18 are detected in 59.8% and 13.2% of the carcinoma cases respectively.



Optimizing CyaA translocation for its use in biotechnological applications as enhanced antigen delivery vehicle

Project PTR n° 502-2022



### SCIENTIFIC COORDINATOR

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# **PROJECT SUMMARY**



# **KEYWORDS**

CyaA, *Bordetella pertussis*, whooping cough, ACD, CaM, P454, ADV, immunization, vaccine, cargo, antigen delivery vehicle, circular dichroism, in *sillico*, double cysteine mutation, disulfide bridge, Artificial Intelligence, protein confinement, simulation



#### THE KEY ROLE OF CYAA IN WHOOPING COUGH

Whooping cough is a respiratory disease caused by the bacteria Bordetella pertussis. Infections number by B. pertussis is still increasing, making whooping cough a major concern of public health. A key actor of the virulence of Bordetella is its Adenylate Cyclase toxin (ACT, known as CyaA) which plays an important role in the early stages of respiratory tract colonization by the bacteria. Indeed, CyaA allows the invasion of the human cells, called cell intoxication. Thus, this intoxication process leads to cell death, which causes whooping cough symptoms.

#### THE CyaA TOXIN: A POWERFUL BACTERIA SYSTEM

CyaA toxin is a unique system of crossing of the host cells' plasmic membrane. Due to its efficiency to make bacteria target the immune cells, the CyaA toxin, if experimentally reshaped, could be transformed in a powerful vaccination tool. Indeed, two clinical studies use CyaA-based vaccination strategy: for cancer immunotherapy (Theravac) and HPVassociated cervical tumors (ProCervix). Even though, the **CyaA toxin properties need to be optimized for a broader** use in therapeutic strategies.

Following this objective, the project coordinator and his team has recently demonstrated that one component of the CyaA toxin, the peptide P454, is a crucial actor for the membrane crossing. P454 can destabilize the host's plasma membranes, passes across it, and then interacts with an intracellular element: the calmodulin (CaM). Those properties turn P454 as a good candidate to investigate for optimization of CyaA translocation properties to enhance therapeutical strategies based on it.

### **THE PROJECT**

The main objective of this project is to **optimize CyaA for its use in therapeutic approaches**. This project is feasible thanks to the tools developed recently by the teams: one based on CyaA properties, and a protocol involving Artificial Intelligence added to molecular confinement in physiological conditions.

First, the project will use computational approaches to test the **impact of different genetic mutations on the specific part of CyaA involved in host membrane interaction**. The project will show up **optimum mutants** allowing the better interaction between CyaA and targeted cells. Those mutants will be challenged by experimental approaches in cell model.

Altogether, these results lead to the test of a **new optimized generation of CyaA-based therapeutic tools**.

According to World Health Organization whooping cough affects 24 million children under the age of 5 worldwide per year.



# Effect of mood states on olfactory system neural connectome Project PTR n° 511-2022



# SCIENTIFIC COORDINATOR

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# PASTEUR NETWORK COLLABORATORS

Dr Jean-Baptiste MASSON, Institut Pasteur Paris Dr Jean-Yves TINEVEZ, Institut Pasteur Paris

# **PROJECT SUMMARY**

The use of animal model is applied in accordance with regulatory requirements, the "3 Rs" rule described in Directive 2010/63/EU.



# **KEYWORDS**

Depression, olfaction, Neural architecture, microscopy, machine learning, Virtual Reality



#### MAJOR DEPRESSIVE DISORDERS

Major depressive disorder is the largest contributor of disability worldwide, so there is an urge to better understand the neuronal circuits involved in those diseases Understanding how the brain processes and updates sensory precepts is a key challenge to better clarify several basic and clinical issues. For instance, the etiology of major depressive disorder is believed to be multifactorial. In fact, sensory alterations are known to be key element of psychiatric disorders in general, such as attention-deficit/hyperactivity disorders, bipolar disorders, or schizophrenia. Knowing that sensory perception is strongly shaped by the properties of the connections (connectome) formed between sensory systems and the rest of the brain, a strong analyze the sensitive connectome should lead to new therapeutical routes.

#### SENSITIVE SYSTEMS AND PSYCHIATRIC/PSYCHOLOGIC DISORDERS

Perception is the set of mental processes that enable an individual to recognize, organize and interpret intellectual, sensory and emotional data in a logical or meaningful way. **Perception disruption can have dramatic behavioral consequences** for animals, comparable with important human mental pathologies. In the specific case of **olfactive impairment**, it has been demonstrated in human that it goes with **depressive symptoms**. Moreover, the project collaborators have previously demonstrated that in a **mouse model of depression/anxiety that neurons that grow in their adult life have a key role in olfactory learning**. But mechanisms linking depression state and alteration of olfactory system remain poorly known.

#### THE PROJECT

The project focus first on a better understanding of the neuronal connections involved in olfactory perception (olfactory connectome). The project will trace the communication ways between the neurons by microscopy. This work will allow the identification and comparison of the connectivity of olfactory neurons in physiological and pathological contexts.

In parallel we will build a complete ecosystem of **bioinformatics tools to more efficiently analyze microscopic acquisitions.** A self-supervised learning (SSL) pipeline with a virtual reality interface will be developed **to accelerate and enhance the robustness of acquisition and analysis.** 

The project will identify the partners of olfactory neurons and characterize the alterations of this partnership in a depressed state. The tools developed will provide a new way of processing and analyzing the different remodeling of neuronal circuits involved in various psychiatric disorders. Overall, the results of the project will pave the way for new and better targeted therapeutic strategies.

5% of adults suffer from depression worldwide (WHO)

According to a CNRS 2015 study, around one in ten French people suffer from olfactive impairment



# Neuronal excitability as a neurophysiological substrate for sensitivity to sounds

Project PTR 520-2022



#### SCIENTIFIC COORDINATOR

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### **PASTEUR NETWORK COLLABORATORS**

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# **PROJECT SUMMARY**



#### **KEYWORDS**

Diagnosis, Biomarkers, monitoring, model for research, ASD, autism, hyperacusis, mouse model, gerbil model, sensory disorder, hyperexcitability, sound roughness, anxiety, hyperresponsiveness, hyperexcitability, neural circuit

# HYPERSENSITIVITY TO SOUNDS

There exists dramatic differences in the way individuals respond to sound. In hypersensitivity, people suffer from sounds that may seem quite ordinary for others. There are several types of hypersensitivity, including misophonia (strong aversion to certain human-produced sounds), phonobia (an excessive fear of sound) and hyperacusis (low tolerance or hypersensitivity to everyday sounds). Typically developed individuals (TD) and individuals with Autistic Spectrum Disorders (ASD) may have unusual sensory sensitivity to sound. This condition can be disabling, leading to negative sensations and emotions, heightened anxiety and social isolation, ultimately affecting both professionnal and personnal lifes. Little is known about the neuronal circuits and mechanisms underlying hyperacusis. In an increasingly noisy modern world, it is essential to identify the neural causes and consequences of this debilating sensory disorder.

### HYPERACUSIS IN TD AND ASD

Hyperacusis is the most common form of sound hypersensitivity in the general population. However, little is known about the physiopathology of hypersensitivity in both TD and ASD people. Interestingly, aversion to rough sounds (such as alarm signals) is enhanced in individuals with ASD and in hyperresponsive individuals. In addition, anxiety increases behavioural and neural responses to rough sounds in TD people. The consortium previously evidenced enhanced sensitivity to rough sounds in mice models, and demonstrated that activity in the primary auditory cortex correlates with sensitivity. Thus, these evidence point to a relationship between sound hyperresponsiveness and neural hyperexcitability. As such, sounds roughness response constitutes a promising starting point for studying the mechanisms underlying sound aversion as featured in hyperacusis.

# **THE PROJECT**

The consortium hypothesis is that the basic mechanisms underlying hyperacusis are shared between individuals withTD and ASD. The aim of this project is to **investigate the relationship between responsiveness to sounds and the excitability of neural circuits.** Using rough, aversive sounds they will probe the putative links between behavioral, emotional and neural responses in TD and ASD individuals, as well as in gerbil and mouse models. This project will provide a **new avenue for developing effective diagnostic tools for hyperacusis.** In addition, this approach constitutes a new entry point for studying brain impairments in ASD. For the first time, this research allows testing, in an enriched environment, direct correlations between the animal model daily activities, physiological state and sound-induced alterations.



It is estimated that 2-15% of people experience hyperacusis.

60 to 96% of people with ASD report unusual sensitivity to sensory stimuli.



# Targeting the peptidoglycan assembly complexes

# Project PTR n° 526-2022



#### SCIENTIFIC COORDINATOR

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# **PROJECT SUMMARY**



\* HTRF: Homogeneous Time Resolved Fluorescence

\* BRET: Bioluminescence Resonance Energy Transfer

#### **KEYWORDS**

Macromolecular complexes, nucleic acids, proteins, carbohydrates, vaccines, antimicrobial, peptidoglycan, PGN, gastric ulcer, Helicobacter pylori, bacteria, cell envelope, antibiotic resistance, Penicillin-binding protein, PBP, protein:protein interface, MreC



# **BACTERIA'S BURDEN OVER HUMAN**

With the increase of **antimicrobial resistant pathogens** number, there is an urgent need of new therapeutical strategies. Indeed, in 2019, on a study covering 471 milion of patients worldwide, it was estimated that at least **4·95 million deaths** was linked to an antimicrobial resistant pathogen, including **1.27 milion attribuable to bacteria resistant to antibiotics.** 

# **BACTERIA CORE PROTEINS: AN ACHILLE HEEL ?**

**Bacteria share common mechanisms** that are essential for their survival. For example, the mechanisms dedicated to the multiplication and shaping of their cells. The assembly of proteins to constitute their cell wall is particularly interesting to study because all interactions of bacteria with their environment depend on it.

The construction of the bacteria enveloppe need pecular proteins assembly, such as the Penicillin-binding protein named PBP2 with the structural protein MreC: it forms the **complex PBP2:MreC**. Interestingly, when this complex formation is targeted in the bacterium *Helicobacter pylori\**, it kills the bacterium *in vitro*. So this complex assembly seems to be an ideal target to find new antibacterial agent.

\*bacterium colonizing the stomach of about half of the human population worldwide.

# **THE PROJECT**

This project aims to **develop a brand new antimicrobial strategies** targetting the PBP2:MreC complex assembly. To reach this objective, the teams will explore further the complex itself computationnaly and on bench. Then they will screen bank of molecule to develop inhibitors of the PBP2:MreC complex assembly. This project will bring a **new rational innovative way to screen and discover new antibiotic candidates** targeting biosynthesis of proteins of the bacteria cell wall. This approaches has the advantage to target essential proteins for the bactreria which are directly in contact of its environment.

In 2017, WHO counts 12 antibiotic resistant bacteria families as priority area of new treatment research

In France, 15 to 30% of the population is thought to be contaminated by Helicobacter pylori,



# Mechanisms of Heteroresistance leading to antifungal treatment failure

Project PTR n° 530-2022



#### SCIENTIFIC COORDINATOR

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# **PROJECT SUMMARY**



# **KEYWORDS**

mycology, fungal pathogens, *Candida albicans,* candidiasis, antifungal resistance, azole heteroresistance, molecular genetics, microfluidics, population heterogeneity



# THE IMPACT OF FUNGAL INFECTIONS ON GLOBAL HUMAN HEALTH

**Fungi are ubiquitous in the environment**: the fungal kingdom has about 2.8 million species, making it the second largest group in the living world. Fungi are known to survive in extreme conditions and are natural colonizers of the human body (skin, oral cavity, respiratory, gastrointestinal and urogenital tracts). Fungi represent 1 to 2% of the total microbial biomass of the human microbiota. Some fungi evolved alongside with humans and are important players in human health, including as modulators of the adaptive immune response. However, this commensal relationship can also turn into invasive opportunistic disease. Indeed, fungal infections cause over 1.7 million deaths per year worldwide, as many as malaria or tuberculosis.

# **CANDIDA ALBICANS INFECTIONS**

Commensal fungi include *Candida albicans*, a species commonly found in the digestive system and on the skin of humans. However, they are pathogenic and life-threatening in certain situations. *C. albicans* can make its way through the intestinal wall, enter the bloodstream and invade the entire body. This fungus is the **most common causative agent of fungal bloodstream infections and is associated with a high mortality rate** (50%), even with treatment. The incidence of invasive fungal infections is increasing and there is no vaccine against these pathogens. Currently, there are only 3 classes of antifungal drugs, and resistance has been reported for all of them. The challenge is further compounded by the fact that strains which appear to respond to treatment in the laboratory can persist during treatment in the host. The mechanisms behind *C. albicans* clinical persistence remain unknown but understanding them is critical for developing improved antifungal strategies.

### **THE PROJECT**

This project aims to **understand the mechanistic basis of** *C. albicans* **heteroresistance to antifungal drugs**. It is known that fungi can alter the copy number of parts of their genome and this ability can help them adapt to stressful environments. The hypothesis of this project is that cell to cell heterogeneity within fungal populations enables the formation of rare cells that have altered genome copy numbers and these cells are highly resistant to antifungal drugs. In addition, this project will explore the impact of genome instability factors on the ability of *C. albicans* to become resistant. This project will provide new predictors of antifungal resistance and provide important insights on the management of fungal infections.

There are >184 species of fungi colonizing in the human gut.

It is estimated that there are 1.7 million deaths per year from fungal infections.



# Mechanics of Inviding Glioblastoma Cells

Project PTR 548-2022



#### SCIENTIFIC COORDINATOR

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# **PROJECT SUMMARY**



# **KEYWORDS**

Glioblastoma cells, glial cells, cancer, cell junction, cell-adhesion, cell communication, 3D cell culture, microscopy, cell migration, cell invasion, intermediate filaments, cytoskeleton



# THE GLIOBLASTOMA MULTIFORME (GBM)

Gliomas are the most common malignant brain tumours that originate from glial cells. These cells represent 90% of the brain cells and their role is to create a favorable environment for the neurons (protection against pathogens, cell renewal, nutrition...). Gliomas are also the 2nd most frequent and deadliest tumours in children. Amoung Gliomas, the Glioblastoma multiforme (GBM) is the most frequent one. GBM is is a fast-growing and aggressive brain tumor and, despite treatments (surgery, chemotherapy, radiotherapy), only 0.05 to 4.7% of patients survive for more than 5 years after diagnosis. But current diagnosis are not sufficient to predict the disease progression. Hence, there is an urgent need for a better characterization of GBM invasion and the development of new therapeutical approaches.

# FOCUS ON THE TOPIC

Today, due to the rapidity and invasive character of the GBM, prognosis is challenging. The cell invasion consists in cell migration through 3D complex and changing microenvironments. It is a physiological phenomenon during the development and immune answer for instance, but in case of cancers, the cell invasion is dysregulated and leads to the tumours and metastases production. **GBM cells, which migrate away from the tumor core, evade surgery, radiotherapy, making tumor invasion the leading cause of death.** A better understanding of the invasion mechanism is crucial to fight against this kind of cancers. Identification of molecular markers and new therapeuthical routes are needed to analyse and prevent the cells deadlious invasion occuring in cancers such as GBM.

# THE PROJECT

This project aims to **develop a new technique combining new synthetic 3D cell culture scaffolds**, a cell model, microscopy, and different techniques to measure the passive (elasticity, viscosity, deformation...) and active (molecule production, degradability, environmental sensitivity...) mechanisms involved in the GBM cell model during the invasion phenomenon. Their hypothesis is the keyrole that mays play the intermediate filaments from the cytoskeleton. Preliminary results tend to demonstrate that the composition of those structures is crucial for the invasion phenomenon. By analyzing in a precise and interdisciplinary way the mechanisms involved in tumoral cells invasion, the consortium will **provide a new route to better understand tumor behavior and improve patient prognosis.** Moreover, the technological pipeline developed here for 2D and 3D cell analysis is highly transposable to other biological fields such as Embrology, or Parasitology for example. The Gliomas are rare cancers, with an incidence of 4.67 to 5.73 cases per 100,000 individuals worldwide (age -adjusted)

Response rates to chemotherapy have rarely exceeded 5% and treatment failures usually occur after 3 months of treatment.

**INCENTIVE RESEARCH PROGRAMS - SPAIS**


# Comprehensive profiling of human and mouse metastasis-infiltrating ILC

Project PTR n° 550-2022



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# **PROJECT SUMMARY**



### **KEYWORDS**

Transcriptomics, Innate immunity, tumour, immunology, liver, lung, ILC1, NK cell, cancer, metastasis, single cell, RNA-seq, cell-sorting



### THE DISEASE

Cancer is a large group of diseases that can affect any part of the body. In 2020, cancer was responsible of 10 million death which put it as one of the major death causes worldwide.

Cancers are characterized notably by the rapid creation of abnormal cells that grow beyond their usual boundaries and invade parts of the body they belong to. The next step of the disease is abnormal cancerous cells spread to other organs where they form new abnormal cells mass: metastasis. This process represents the major cause of death in cancer patients. More than 5% of patients show liver metastases at the time of primary cancer diagnosis, a feature associated with poor prognosis and low 1-year survival rates.

## **METASTASIS AND IMMUNE CELLS RELATIONSHIPS**

Metastases develop mainly in the lungs, liver, bones and brain. Some immune cells can infiltrate the metastases and are cell-killers: the Natural Killer (NK), circulating in blood, and the Innate Lymphoid Cells 1 (ILC1) which stay in tissues. NK naturally have anti-tumourous properties, and are the core target of some immunotherapies. ILC1, in contrast to NK cells, do not recirculate in the body but reside in tissues and could represent a first line of defense against metastatic seeding. Currently, the understanding of the heterogeneity and function of NK cells and ILC populations that infiltrate metastases is limited, yet, knowledge thereof could be key for the development of optimized treatments.. Better understanding of their abilities as cell-killers and metastasis invaders would be game changer for developping new anticancerous therapies.

#### THE PROJECT

The project aims to better understand the role of NK and ILC1 in the neutralization of metastases. To do so, the teams will use mouse models with initial colorectal cancer or melanoma with lung and liver metastases. They will identify the role of NK and ILC1 in the different stages of the metastasis process, notably by analyzing the evolution of their DNA transcription activity. This will allow to identify which factors of NK and ILC1 are used during their invasion in metastasis. In this way, the project will provide a comprehensive picture of the evolution of NK and ICL antitumor activity across metastases and identify turning points in tumor progression and neutralization. This study will provide novel insights into pathways involved in the metastatic process and the scientific framework to design preventive and therapeutic strategies for the treatment of metastatic patients.

In 2020, lung, liver, colon and rectal cancer were the most lethal ones, causing together 3.5 million deaths worldwide.

Tumoral cells as well as immune cells in the tumor environment are driven by processes leading to huge heterogeneity (mutations, epigenetics...)



# Targeting mitochondrial membrane integrity in Chagas Disease

#### Project PTR n° 558-2022



#### SCIENTIFIC COORDINATOR

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## **PROJECT SUMMARY**



#### **KEYWORDS**

Chagas disease, *Trypanosoma cruzi*, mitochondria, metabolism, parasite, immunity, physiopathology, mice model, organelle, cell trafficking, cell model, dilated cardiomyopathy, Chronic Chagasic Cardiomyopathy Disease, preclinical, inner membrane



### THE DISEASE

**Chagas disease (CD)**, also known as **American trypanosomiasis**, is a zoonotic and life-threatening tropical illness caused by the **parasite** *Trypanosoma cruzi*. Human transmission can be congenital, food-borne, made by insect bite, blood transfusion or organ transplant for instance. CD is characterized by a first acute phase with mild, unspecific or absent symptoms and may continue in a chronic phase with serious complications, such as cardiac dysfunction (30% of the cases), digestive and/or neurological alterations (10%). Today, Chronic Chagasic Cardiomyopathy (CCC) represents the most frequent non-iscemic cardiomyopathy in Latin America, and there is no treatment for this disease. However the physiopathological mechanisms responsible for disease manifestations and progression remain poorly known.

# THE MITOCHONDRIA AND INFLAMMATORY RESPONSE

Mitochondria are key components of cells. They are organelles that allow energy production and drive response to cellular stress. Moreover, mitochondria possess their own DNA (mtDNA) that has been shown to modulate the inflammatory response. Mitochondria are surrounded by a double membrane (internal and external) whose integrity is a determining element of cell survival. Indeed, pathological remodeling of mitochondrial membranes caused by genetic, non-infectious lesions can trigger the development of dilated cardiomyopathy with key features reminiscient of Chagas disease (CD). Given these similarities, and the fact that the molecular mechanisms of chronic Chagas cardiomyopathy caused by *Tripanosoma cruzi* infection are unknown, the physiology of this organelle is a prime candidate to study to better understand Chagas Disease.

#### THE PROJECT

This project aims to better understand the potential role of mitochondrial physiology in the chronic cardiomyopathy of Chagas disease. To do so, the team will generate mouse and cell models mimicking Chagas cardiomyopathy. Moreover, they will target mutations in mitochondrial inner membrane regulators on those models, which will either strengthen or weaken the integrity of the inner membrane and assess the outcome of infection. Studying the impact in cell and trasgenic mouse models under basal conditions and in the case of *Trypanosoma cruzi* infection has the potential to identify new therapeutic targets to cure Chagas cardiomyopathy. Moreover, this project will formalize and unite a trio of Pasteur Network teams from France and Uruguay allowing bilateral transfers of skills and knowledge on pre-clinical approaches dedicated to the expertise building on an emerging infectious disease that has so far been largely neglected.

Chagas Disease is endemic in 21 countries of continental Latin America.

1/3<sup>rd</sup> of the volume of cardiac cells (cardiomyocytes) is composed of mitochondria



Integrative approach to combat antibiotic resistance by targeting protein-protein interactions essential to bacterial macromolecular machinery

Project PTR n° 566-2022



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#### **PROJECT SUMMARY**



## **KEYWORDS**

Antimicrobial resistance, AMR, antibiotic, therapeutical strategy, divisome, protein-protein interaction, PPI, Fr-PPI-Chem Library, Bacterial Two Hybrid Screening Technology, BACTH, *Escherichia Coli, E. Coli* 



#### ANTIBIOTICS OVER-USE AND CONSEQUENCES

Antibiotics are known to save millions of life per year since its commercialization in the 40s. Even through, because of their excessive use, **bacteria evolve quickly and oppose an important antimicrobial resistance.** Infections in humans and animals caused by resistant bacteria are harder to treat, as they don't answer to antibiotic treatments anymore. In extreme very rare cases, bacteria can be resistant to all available antibiotics used in humans. In 2019, it was estimated that **antimicrobial resistance was responsible for 1.3 million of death worldwide.** 

#### THE URGE TO FIGHT AGAINST ANTIMICROBIAL RESISTANCE

Human and financial cost of the antimicrobial resistance (AMR) keeps rising. It is estimated that **10 million people could die annually from antimicrobial resistance.** But on other hand, it is impossible to completely stop the antibiotics treatments use. In this context, the WHO considers that AMR is one of the biggest threats to global health, food security and development.

Bacteria resistance can consist in various molecular mechanisms such as reduced access to the antibiotic target (reduced membrane permeability...), direct change on this target so antibiotics doesn't recognize it anymore, and genetic mutation of major importance. It is necessary to better understand the bacteria core mechanisms to find new routes to create innovative treatments, avoiding the antimicrobial resistance as much as possible.

## **THE PROJECT**

The project aims to use innovative pathways to fight bacteria while avoiding the occurrence of AMR. The strategy is to target the protein-protein interaction of bacteria using models in vivo and in silico. The teams want to build a comprehensive pipeline to improve the identification of drugs that can shut down a specific protein-protein interactions so that the bacterial structure is disrupted and the bacteria die. The project will build on existing libraries of molecules dedicated to protein-protein interactions: the fragment library hosted on the Institut Pasteur campus in Paris and the Fr-PPI-Chem. Using an E.coli model, they will screen the impact of the molecules present in these libraries in order to find the most effective and the least likely to develop resistance. The screening methodology is already developed thanks to one team of the consortium. In addition, thanks to the protocol pipeline that the teams will build, they will be able to extend it to the screening of millions of commercially available compounds. By accelerating the screening of effective and less AMR-prone drugs, the teams will create a robust tool against one of the most challenging health problems in recent years.

In France, physicians wrote 705 prescriptions per 1,000 inhabitants for the year 2021.

By 2050, it is expected that antimicrobial resistance will be responsible of 569 million extra hospital days annually.



# APOBEC3CI188 mutator enzyme, a genetic predisposition marker for liver cancer

#### Project PTR n° 588-2022



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#### **PROJECT SUMMARY**



#### **KEYWORDS**

Genetic predisposition, liver cancer, A3C<sub>I188</sub>, APOBEC3<sub>CI188</sub>, enzyme mutator, cytidine deaminase, reverse genetics, genome editing, recombination, molecular genetics, cancer, diagnosis, biomarker, Hepatocellular carcinoma, HCC, variant, mouse model, sequencing,



## **HEPATOCELLULAR CARCINOMA**

Hepatocellular carcinoma (HCC) is a "primary" liver cancer (PLC), a highly heterogeneous disease that develops from liver cells. HCC is the most common form of liver cancer (~90% of cases). It is the 5<sup>th</sup> most common cause of cancer worldwide and the 3<sup>rd</sup> most common cause of death from cancer. Hepatocellular carcinoma generally occurs in a liver that is already damaged in the context of a chronic disease involving unsolved inflammation and cytokine dysregulation. The most common causes are hepatitis B virus, hepatitis C virus, alcohol intoxication, and non-alcoholic steatohepatitis (NASH). In most sub-Saharan African countries, curative treatment of primary liver cancers by surgery is not practiced due to a lack of resources. However, surgery material from patients is the golden standard material for molecular mechanisms analysis involved in PLC. Thus, there is a growing gap between the available knowledge on PLC and its application to sub-Saharan populations.

# **GENETIC PREDISPOSITION TO HCC**

Approximately 25% of HCC tumors present actionable mutations, but many driver genes and prognosis markers in HCC remain to be identified. The teams of this project have demonstrated that the variant enzyme A3CI188, mays be involved in cancer. This enzyme is particularly important for the transmission of genetic information as it edits mRNA.  $A3C_{I188}$  is a variant of the *APOBEC3C* enzyme whose initial activity is editing genetic material to allow protein diversity. However, when variant  $A3C_{I188}$  is present, this activity is increased and causes a higher number of DNA mutations that can lead to cancer. Moreover, variant  $A3C_{I188}$  is present at about 10% frequency in diverse populations throughout Sub-Saharan Africa.  $A3C_{I188}$  represents a potential Achille's heel for those populations where it might play a role in infection-associated tumors.

# **THE PROJECT**

The project aims to understand the role of variant *A3Cl188* in human cancerogenesis. Understanding the mechanisms of this variant will lead to new relevant biomarkers and prevention tools for Hepatocellular carcinoma. This project will also build a global vision of the mutation spectrum of PLC and co-carcinogenic factors in African patients. Moreover , by developing a new mouse model, this project will bring new knowledge on the impact of genome editing on health and development

By 2030, there will be a 35% increase in the future number of new cases of PLC annually, compared to 2005 (Study over 30 countries worldwide)

50–60% of patients with HCC are estimated to be exposed to systemic therapies in their lifespan



Origins and dynamics of oral dysbiosis associated with enteric dysfunction. Preventive intervention in Central African infants.

Project PTR n° 596-2022



#### SCIENTIFIC COORDINATOR

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#### **PROJECT SUMMARY**



#### **KEYWORDS**

Metagenomics, epidemiology, surveillance, environmental enteric dysfunction, EED microbiota, oral dysbiosis, MetaHiC, public health, chronic infection, neonatal infection, stunting,



### THE DISEASE

Environmental enteric dysfunction (EED) is an acquired small intestine disorder characterized by a chronic inflammatory condition. This pathology is caused by the disruption of the epithelial intestinal barrier, leading to poor nutrient absorption. EED is intimately associated with child undernutrition, unsafe water, and poor sanitation and hygiene conditions, which contribute to stunted growth in children, impaired neurocognitive development, and reduced response to oral vaccines. Due to a lack of models, the precise mechanisms involved in this disease are poorly known. Precedent attempts to treat EED- related stunting by nutritional treatments weren't conclusive: there is a need for new approaches.

#### FOCUS ON THE TOPIC

In a precedent project, the consortium revealed that stunting was associated with small intestinal bacterial overgrowth, dominated by bacteria that normally reside in the oropharyngeal cavity and are associated with small intestinal inflammation and decreased lipids absorption. Moreover, another study demonstrated the presence of oral dysbiosis in stunted children. Those results bring a new point of view on the stunting etiology and put the microbiota in the position of a good target and biomarker of stunting and EED risk. In this context, it is necessary to unravel the oral dysbiosis's origins and the curative measures' impact on it (hygiene, prevention...) to better understand how EED occurs in patients.

#### THE PROJECT

The project is a continuation of the Afribiota-1 project, conducted by the same consortium. Thus, the project capitalizes on the data, collaborations, and cohorts obtained previously, which is a considerable asset for the feasibility of the project.

The project aims to better understand the oral microbiota in the context of stunting in EED through the first use of the MetaHiC sequencing technique on samples directly from the field. MetaHiC will overcome the technological limitations encountered to date that have hampered such an approach. The teams will analyze the ecology of the oral microbiota, its antimicrobial resistance genes and genomic acquisitions, its dynamics, and its persistence. To develop a preventive approach to oral dysbiosis and EED, the consortium will conduct a clinical trial measuring the impact of an oral hygiene intervention on infant growth and the incidence of stunting.

To date 1 in every 4 (23%) children aged < 5 years is stunted worldwide.

In healthy human, it is estimated that some  $10^{14}$ (100 trillion) bacteria live in the intestine.