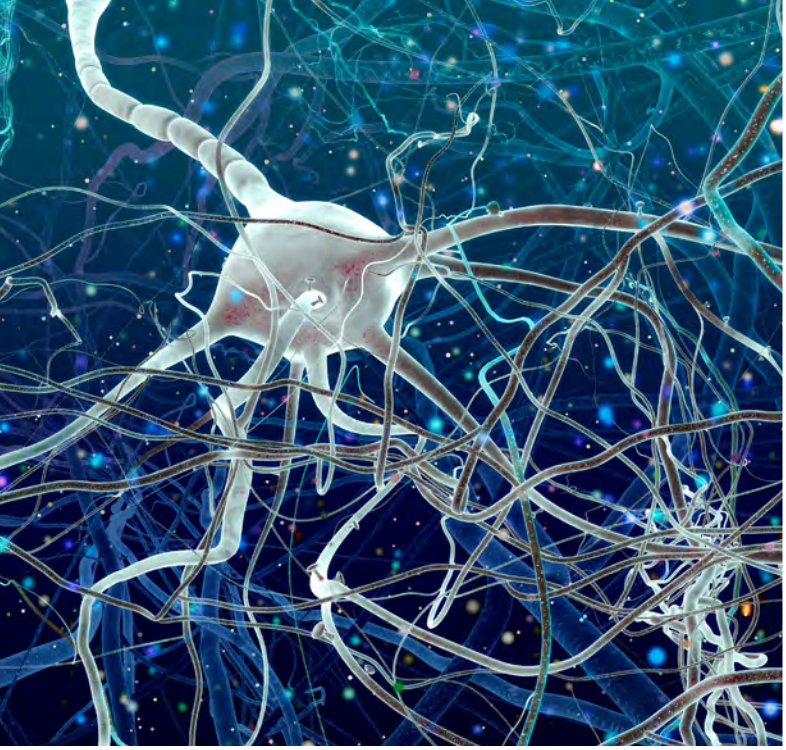
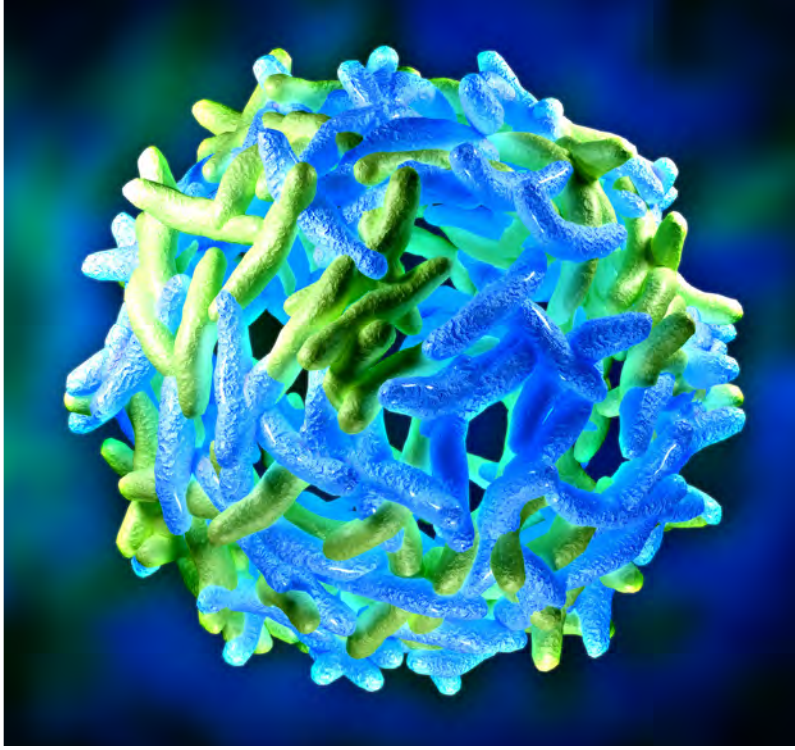
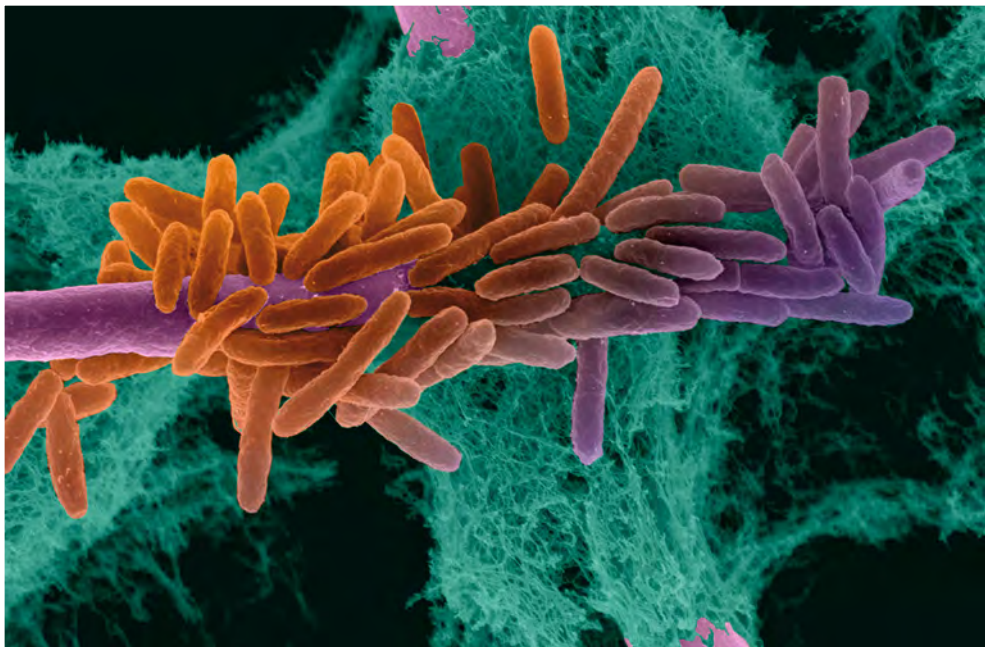




DEPARTMENT FOR SCIENTIFIC PROGRAMMING AND INCENTIVE ACTIONS

Overview of Incentive Research Programs

Updated February 2021



INSTITUT PASTEUR

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 Institut Pasteur International Network (IPIN), a global research community composed of 32 institutes located in 25 countries and spread across five continents. IPIN represents a unique asset in the world for interdisciplinary biomedical research and education.

The newly created Department for Scientific Programming and Incentive Actions (SPAIS), directly attached to the Scientific Direction, is in charge of launching and supporting a series of specific research programs called “Incentive Programs”, notably by using Institut Pasteur seed funding for the early-stage phases. These programs serve as tools for steering and deploying the Institut Pasteur’s scientific vision.

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 IPIN members: **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 calls for proposals follow a well-defined process, from project submission to selection. Submissions are made online through a dedicated platform that establishes a direct link between scientists from the Institut Pasteur and IPIN, 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:

- In the first part, **an exhaustive list of these Incentive Programs** funded under the 2014, 2015, 2016, 2017, 2019 and 2020 calls is shown alongside a topic map.
- In the second part, **short summaries of 90 winning Incentive Programs** are presented separately.

List of funded Incentive Programs from 2014 to 2020

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 inflammatory factors as potential candidates / Eliette Touati – Microbiology Department – Institut Pasteur (Paris)

-ACIP 13-2015: The impact of micro-RNAs and inflammatory 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?: Influence 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 / **T**uberculosis **I**dentification and **D**rug **R**esistance by **A**bsen**C**e of **E**xpression) / 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 (**A**nti-**C**ancer **I**nnovant **P**olytherapy)) / 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 flaccid 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 flu (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 - Laboratório de Genômica Funcional e Bioinformática - Fiocruz (Brazil)

-ACIP 363-2020: Investigating the flea 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)

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 inflammasome 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^{R}) / 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 γ-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 influenza 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 flaviviral 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 inflammatory 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-a-chip (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 microfluidics (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 [2]

-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) [1]

-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 Receptor-expressing 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
[2]: 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)

Mapping of the Incentive Programs: infectious diseases

	Basic research	Epidemiology	Prevention/Surveillance	In vitro diagnostics/ Imaging
Bacteria	**ACIP 06-2014** *M. tuberculosis*/ immune mechanisms/ latent infection **ACIP 10-2015** *H. pylori*/ inflammatory process/ mitochondria alteration **ACIP 358-2020** Polymyxin resistance/ genes/ structural studies **ACIP 363-2020** *Yersinia pesti*/ bubonic plague/ Madagascar **PTR 481-2014** Acute encephalitis syndrome **PTR 494-2014** *Helicobacter*/ gastric cancer/ evolution/ host adaptation **PTR 510-2014** *Bacillus cereus* strains/ genomic diversity/ virulence factors **PTR 521-2015** *Listeria*/ microRNAs/ regulation of bacterial infection **PTR 540-2015** bacterial GABA/ host-bacteria signaling/ host bacterial interaction **PTR 558-2015** ESBL/ newborns/ antibiotic resistance genes/ resistance strains **PTR 08-2016** Malnutrition-PEE/ immunological changes/ explain the altered response to live-oral vaccines **PTR 18-2016** Allergic asthma/ host-bacteria interaction/ immuno-pathological mechanisms **PTR 22-2016** *Shigella*/ *M. tuberculosis*/ interaction bacteria-host/ invasion mechanism/ pathogenicity **PTR 24-2016** *Shigella*/ *EIEC*/ virulent phenotype/ genome arrangement **PTR 39-2016** *Negativicutes*/ gut homeostasie or dysbiosis **PTR 43-2016** *P. aeruginosa*/ characterization of ExoY **PTR 30-2017** *Leptospira*/ virulence factors **PTR 66-2017** *Leptospira*/ immune response **PTR 73-2017** *H. pylori*/ trafficking **PTR 91-2017** Malnutrition-PEE/ gut microbiota **PTR 166-2019** *Bordetella pertussis*/ CyaA toxin/ structure **PTR 232-2019** *Shigella flexeneri*/ mechanical forces/ gut/ microbiome **PTR 245-2019** Gram-negative bacteria/ genes/ aminoglycosides/ antibiotic resistance **PTR 310-2020** *Leptospira*/ virulence mechanisms/ immunity **PTR 332-2020** *H. pylori*/ *E. coli* / bacterial infections and cancer development / DNA methylation **PTR 395-2020** *Legionella pneumophila*/ LpDot1/ functional and structural studies	**ACIP 14-2014** *Ksebsiella pneumaniae*/ multidrug resistant clones/ hypervirulent clones/ intestinal carriage **ACIP 399-2020** Diarrheal diseases/ risk factor/ children/ French Guiana/ Central African Republic/ France **PTR 558-2015** Microbiome composition/ AMR **PTR 91-2017** Malnutrition-PEE/ gut microbiota composition **PTR 303-2020** *Enterobacteriaceae*/ ESBL-PE/ acquisition/ newborns	**ACIP 03-2014** Invasive meningococcal infections/ meningococci of group B/ genetic diversity/ antibiotic sensitivity/ Morocco/ Algeria **ACIP 363-2020** Plague/ fleas/ rodents/ emergence/ Madagascar **ACIP 399-2020** Diarrheal diseases/ Vitamin C/ children **PTR 558-2015** ESBL/ design strategies/ prevent neonatal colonization **PTR 08-2016** Malnutrition-PEE **PTR 18-2016** Allergic asthma/ new perspectives on prevention **PTR 91-2017** Malnutrition-PEE/ gut microbiota **PTR 303-2020** *Enterobacteriaceae*/ ESBL-PE/ newborns	**ACIP 10-2015** *H. pylori*/ non-invasive test/ biomarkers/ prevention detection **ACIP 03-2016** *M. tuberculosis*/ diagnostic platform/ drug sensitivity-resistance **PTR 481-2014** Acute encephalitis syndrome/ identification new pathogens **PTR 510-2014** *Bacillus cereus* strains/ rapid methods/ food security context/ virulence factors **PTR 08-2016** PEE/ biomarkers **PTR 183-2019** *M. Tuberculosis*/ Diagnostic platform/ MDR-TB patients
Viruses	**ACIP 11-2014** HCV/ defective genomes/ liver pathogenesis **ACIP 15-2014** Zika/ molecular evolution/ vector competence **ACIP 22-2015** Dengue/ chikungunya/ vectors/ insecticide resistance **ACIP 01-2016** Dengue/ zika/ mosquito microbiome/ vector competence **ACIP 06-2016** Dengue/ genetics/ evolution **ACIP 22-2016** HFMD/ enteroviruses/ genetic diversity/ Africa **ACIP 162-2019** Enteroviruses/ zoonotic transmission **ACIP 221-2019** Dengue/ virus-mosquito-host interactions/ antiviral immunity/ vectors **ACIP 275-2019** EV-D68/ genetic characteristics **ACIP 281-2019** Dengue/ determinants of emergence/ minority serotypes/ genetic evolution/ immunity **ACIP 318-2020** HBV/ cellular nucleases **ACIP 328-2020** Influenza/ Rhumatoid Arthritis/ epitranscriptome **ACIP 403-2020** Rabies/ immunity/ prophylaxis **PTR 481-2014** Acute encephalitis syndrome **PTR 484-2014** Enterovirus E71/ HFMD/ encephalitis/ genetic diversity/ pathogenicity **PTR 489-2014** Chikungunya/ genetic diversity/ evolution/ transmission **PTR 491-2014** Dengue/ genetic diversity/ transmission mechanism/ host-virus interactions/ pathophysiology **PTR 499-2014** Rabies/ new world bat species/ innate immunity/ IFN/ PKR **PTR 505-2014** Hantaviruses/ host-virus relationships/ pathogenicity **PTR 546-2015** Influenza virus/ viral replication/ strain virulence **PTR 10-2016** Arbovirus/ virus-host interactions **PTR 20-2016** Chronic hepatitis B/ cellular mechanisms maintining inflammation **PTR 98-2017** Ebola virus/ functional role of sGP **PTR 161-2019** Enteroviruses/ genetic diversity **PTR 212-2019** Dengue/ protective humoral immune response **PTR 218-2019** Monkeypox/ animal reservoir/ transmission/ risk factors/ ecological factors **PTR 291-2020** Influenza/ muscle stem cells regeneration/ acute systemic pathogen-driven inflammation **PTR 353-2020** Foamy viruses/ host-pathogen interaction/ human health/ Env protein structure	**ACIP 03-2015** Hepatitis E virus/ Ab prevalence rates/ HEV RNA detection **ACIP 22-2016** HFMD/ Africa **ACIP 275-2019** EV-D68/ Children/ Senegal/ Ivory Coast **PTR 505-2014** Hantaviruses/ seroepidemiology in animals/ Madagascar **PTR 161-2019** Enteroviruses/ Central Africa/ Madagascar **PTR 218-2019** Monkeypox/ risk factors/ Central Africa	**ACIP 09-2014** Dengue/ human and entomological surveillance/ transmission/ virus circulation **ACIP 03-2015** Hepatitis E virus/ national recommendations **ACIP 22-2015** Dengue/ chikungunya/ vector control **ACIP 01-2016** Dengue/ Zika/ vector control/ surveillance **ACIP 22-2016** HFMD/ precautionary measures **ACIP 162-2019** Enteroviruses/ zoonotic transmission/ herd animalas/ wild apes/ Cameroon/ Ivory Coast **ACIP 255-2019** mosquitoe-borne-diseases/ behaviour/ infected mosquitoes/ pathogen emergence **ACIP 275-2019** EV-D68/ Children/ Senegal/ Ivory Coast **ACIP 281-2019** Dengue/ determinants of emergence **PTR 484-2014** Enterovirus E71/ HFMD/ encephalitis/ epidemic risk/ seroprevalence studies **PTR 546-2015** Influenza virus/ tracking risks of pandemics **PTR 528-2015** Yellow fever/ vector competence **PTR 161-2019** Enteroviruses/ Central Africa/ Madagascar **PTR 218-2019** Monkeypox/ Central Africa **PTR 237-2019** Rabies/ Madagascar/ Cambodia/ humans/ animals	**ACIP 04-2014** Arboviruses/ multiplex technology **ACIP 15-2014** Zika / standardization methods **ACIP 255-2019** mosquitoe-borne-diseases/ infected mosquitoes/ behaviour platform **PTR 481-2014** Acute encephalitis syndrome/ identification new pathogens **PTR 20-2016** Chronic hepatitis B/ diagnostic and prognostic biomarkers **PTR 98-2017** Emerging-remerging diseases/ ELISA and dipstick **PTR 218-2019** Monkeypox/ serological assay **PTR 237-2019** Rabies/ non-invasive samples/ field tests **PTR 353-2020** Foamy viruses/ serological assay
Parasites	**ACIP 02-2014** Malaria/ vector **ACIP 41-2017** Malaria/ *A. gambiae*/ *A. coluzzii*/ vector genomic diversity/ transmission **ACIP 76-2017** *T. gondii*/ centrosomes **ACIP 253-2019** Malaria/ Anopheles/ *Asaia*/ vectorial capacity **PTR 490-2014** *P. vivax*/ host-parasite interactions **PTR 496-2014** *Leishmania*/ host-parasite interactions **PTR 535-2015** *P. falciparum*/ artemisinin resistance **PTR 539-2015** *Leishmania*/ host-parasite interactions **PTR 542-2015** *Plasmodium*/ *trypanosoma*/ vectorial capacity/ multi-transmission **PTR 190-2019** Trypanosomatids/ structural studies/ protein synthesis machinery **PTR 232-2019** *Entamoeba histolytica*/ mechanical forces/ gut/ microbiome **PTR 233-2019** *P. falciparum*/ multi drug resistant parasites/ epigenetic processes	**PTR 542-2015** *Plasmodium*/ *trypanosoma*/ transmission risk/ vector control	**ACIP 02-2014** Malaria / vector control **ACIP 253-2019** Malaria/ Anopheles/ *Asaia*/ vectorial capacity	**PTR 535-2015** *P. falciparum*/ artemisinin resistance **PTR 175-2019** Sleeping sickness/ field-adapted method
Fungi	**PTR 529-2015** *Aspergillus fumigatus*/ hydrophobins **PTR 26-2016** Aspergillosis/ drug resistance		**PTR 26-2016** Aspergillosis/ surveillance and management diseases/ Cambodia	

Therapeutic	Vaccine	Biobank/Database	Disease models/Research tools	
PTR 166-2019 *Bordetella pertussis*/ Whooping cough/ CyaA toxin/ inhibitors PTR 310-2020 *Leptospira*/ molecular targets PTR 332-2020 *H. pylori*/ *E. coli* / bacterial infections and cancer development / novel therapeutic strategies PTR 395-2020 *Legionella pneumophila*/ new targets	ACIP 03-2014 Invasive meningococcal infections/ new vaccinal recommandations PTR 08-2016 Malnutrition-PEE/ novel strategies to boost vaccine responses PTR 30-2017 *Leptospira* PTR 52-2017 TB/ prophylactic and therapeutic approaches PTR 66-2017 *Leptospira* PTR 166-2019 *Bordetella pertussis*/ Whooping cough/ CyaA toxin/ protective antigen/ antigen delivery vector PTR 380-2020 *Campylobacter jejuni*/ chemical synthesis/ safe and effective vaccine	ACIP 03-2014 Meningococcal strains/ database ACIP 363-2020 Plague/ flea/ rodents/ Madagascar ACIP 399-2020 Diarrheal diseases/ children/ plasma/ French Guiana/ Central African Republic/ France PTR 30-2017 *Leptospira* strains PTR 303-2020 *Enterobacteriaceae*/ ESBL-PE/ newborns-mothers/ meconium-stool / Madagascar	ACIP 06-2014 *M. tuberculosis*/ rat model/ *Mtb* infection/ screen novel TB vaccine PTR 18-2016 Allergic asthma/ humanized mouse model PTR 22-2016 *Shigella*/ *M. tuberculosis*/ "organ-on-a chip" PTR 43-2016 *P. aeruginosa*/ cell line expressing ExoY PTR 232-2019 *Shigella flexeneri*/ gut-on-chip technology/ software PTR 332-2020 mouse model / compounds screening	**Bacteria**
ACIP 18-2015 Dengue/ yellow fever/ Hepatitis B/ antivirals natural products ACIP 221-2019 Dengue/ therapeutic targets ACIP 318-2020 HBV/ cellular nucleases/ viral DNA synthesis inhibition PTR 546-2015 Influenza virus/ host-virus interactions/ targets PTR 10-2016 Arboviruses/ identification viral and cellular sequence targets PTR 20-2016 Hepatitis B/ optimization PTR 291-2020 Stem cell/ pathogen-driven systemic inflammation interactions/ novel approach	ACIP 06-2016 Dengue/ new antigens ACIP 403-2020 rabies/ PEP recommendations PTR 212-2019 Dengue/ new vaccine strategies	ACIP 09-2014 Dengue/ viral strains/ human samples/ clinical data ACIP 03-2015 Hepatitis E virus/ human samples (plasma)/ swine samples (fecal, liver) ACIP 162-2019 Enteroviruses/ human and herd animal samples (stools) ACIP 221-2019 Dengue/ PBMC and plasma from dengue-positive patients (with mild and severe illness) ACIP 281-2019 Dengue/ DENV-positive persons (serum) ACIP 403-2020 Rabies/ sera/ PBMC/ Madagascar/ Cambodia PTR 161-2019 Enteroviruses/ human samples (nasopharyngeal swab, stools)/ wastewater samples PTR 212-2019 Dengue/ blood of asymptomatic and symptomatic dengue infected individuals PTR 353-2020 Foamy viruses/ human/ blood/ Cameroon	ACIP 09-2014 Dengue/ modeling of transmission ACIP 255-2019 Mosquito-borne-diseases/ behaviour platform PTR 499-2014 Bat immortalized cell lines/ reported cell lines/ antibodies/ rabies PTR 505-2014 Hantaviruses/ reverse genetic techniques/ recombinant proteins/ serological tools PTR 98-2017 Nanobodies/ droplet based microfluidics PTR 291-2020 Single cell sequencing	**Viruses**
ACIP 17-2015 Trypanosomatids/ compounds targeting redox metabolism ACIP 76-2017 *T. gondii*/ candidates targets ACIP 93-2017 *P. falciparum*/ inhibitors PTR 496-2014 *Leishmania*/ targets PTR 539-2015 *Leishmania*/ drugs PTR 190-2019 Trypanosomatids/ inhibitors PTR 233-2019 *P. falciparum*/ new therapeutic strategies/ new antimalarial drug candidates	PTR-490-2014 *P. vivax*/ vaccine PTR 337-2020 *P. falciparum*/ protective antigens/ vaccine		ACIP 41-2017 Malaria/ Anopheles/ PCR genotyping approach ACIP 253-2019 Malaria/ Anopheles/ *Asaia*/ paratransgenesis approach/ vectorial capacity control PTR 535-2015 *P. falciparum*/ K13-specific mAbs/ recombinant proteins PTR 190-2019 Trypanosomatids/ tools PTR 232-2019 *Entamoeba histolytica*/ gut-on-a-chip technology/ software PTR 337-2020 *P. falciparum*/ humanized mice/ immunopeptidomics	**Parasites**
PTR 529-2015 Hydrophobins/ drug delivery formulations		PTR 26-2016 Aspergillosis/ collection of *aspergillus* clinical isolates/ database/ Cambodia		**Fungi**

Mapping of the Incentive Programs: Non communicable diseases

	Basic research	Epidemiology	Prevention/Surveillance	In vitro diagnostics/ Imaging
Central Nervous System	PTR 523-2015 Parkinson's disease/ Alpha synuclein protein PTR 03-2017 Alzheimer's disease/ Schizophrenia/ myasthenic pathologies/ nicotinic acetylcholine receptors PTR 272-2019 Inner ear pathologies/ cochlea/ Sonic-Hedgehog signaling/ morphogenesis PTR 377-2020 Alzheimer's disease/ microglial cells activation PTR 388-2020 Alzheimer's disease/ Schizophrenia/ Parkinson's disease/ Autism/ nicotinic acetylcholine receptors			ACIP 07-2014 Autism/ standard clinical evaluation/ standardized genetic diagnostic/ SNP/ WES PTR 377-2020 Live imaging approaches/ new tools for temporal series of 3D images analysis PTR 388-2020 In vivo brain imaging/ 3 photon technology brain imaging
Neuro-muscular	ACIP 13-2015 Muscular dystrophies/ stem cells/ regulators			ACIP 13-2015 Duchenne and Limb Girdle Muscular Dystrophies/ biomarkers (molecular alterations)
Cancer	ACIP 57-2017 Liver cancers/ metabolic disorders/ gut dysbiosis PTR 24-2017 Cancer/ ubiquitination HP-1 PTR 113-2017 Colorectal cancer/ innate lymphoid cell subsets/ molecular pathways PTR 332-2020 Colorectal cancer and gastric cancer/ bacterial infections and cancer development		ACIP 57-2017 Liver cancers/ measures thwarting liver diseases progression PTR 113-2017 Cancer colorectal/ preventive strategy	
Inflammatory diseases	ACIP 328-2020 Rhumatoid Arthritis/ epitranscriptome PTR 113-2017 Inflammatory bowel disease/ innate lymphoid cell subsets		ACIP 328-2020 Rhumatoid Arthritis/ Flu co-infection PTR 113-2017 Inflammatory bowel disease/ preventive strategy	
other	ACIP 45-2017 Type 2 Diabete /genetics/ gut microbiota/ oxidative stress PTR 483-2014 Usher syndrome/ deafness/ hair cells/ PDZ interactome/ structure-function relationship PTR 24-2017 Ageing/ ubiquitination HP-1 PTR 111-2017 Cockayne syndrome/ mechanism of ageing/ regulators of premature ageing	ACIP 45-2017 Type-2 diabete/ tunisian population	PTR 111-2017 Cockayne syndrome/ mutations	ACIP 45-2017 Type-2 diabete/ biomarkers

Mapping of the Incentive Programs: Not-disease-related

	Basic research	Epidemiology	Prevention/Surveillance	In vitro diagnostics/ Imaging
basic research, biology, genomics,...	PTR 35-2016 Immune response/ inter-individual variability/ genetic and environmental differences			

Therapeutic	Vaccine	Biobank/Database	Disease models/Research tools	
PTR 523-2015 Parkinson's disease/ new targets PTR 03-2017 Alzheimer' disease/ Schizophrenia/ myasthenic pathologies/ nicotinic acetylcholine receptors/ potential drug targets PTR 377-2020 Molecular mechanisms of neuroinflammation/ new therapeutic approach PTR 388-2020 New pharmacotherapy (allosteric modulators)		ACIP 07-2014 Medical and genetic data	PTR 272-2019 Inner ear pathologies/ organoid technology/ mouse PTR 377-2020 Mouse model/ new tools for temporal series of 3D images analysis PTR 388-2020 Rat model/ touchscreen operant platform	**Central Nervous System**
				Neuro-muscular
ACIP 05-2017 Hepatocellular carcinoma/ targets/ anti-cancer drugs from scorpion and snake venoms PTR 113-2017 Cancer colorectal / new small molecules PTR 332-2020 *H. pylori*/ *E. coli*/ bacterial infections and cancer development/ novel therapeutic strategies		ACIP 57-2017 Liver diseases/ human samples (stools/ blood/ liver tissues)	PTR 113-2017 Generation new strain of mouse PTR 332-2020 Mouse model/ compounds screening	**Cancer**
PTR 113-2017 Inflammatory bowel disease/ target identification				**Inflammatory diseases**
PTR 111-2017 Cockayne syndrome/ modulators		ACIP 45-2017 Type-2 diabete/ human samples (fecal and blood) PTR 111-2017 Cockayne syndrome/ skin and muscle biopsies/ blood samples		**other**

Therapeutic	Vaccine	Biobank/Database	Disease models/Research tools	
PTR 35-2016 Immune response/ inter-individual variability/ genetic and environmental differences	PTR 35-2016 Immune response/ individual variability/ new development adjuvants and vaccines			**basic research, biology, genomics,...**

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

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.

International budget for the control and elimination of malaria in 2013: 2,7 billion of dollars.

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.

Morocco was declared malaria-free in 2010 but lately 100 cases per year have been identified.

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.

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 semi-closed 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.

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

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.

> 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.

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

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.

Acid-nucleic based tests are highly specific compare to serological-based ones.

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.

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

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.

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.

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.

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 evaluations. The heterogeneity of patients is due to the fact that 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.

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.

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

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

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.

> 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.

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

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.

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.

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.

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

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).*

PUBLICATIONS:

- *Dupont-Rouzeyrol M, O'Connor O, Calvez E, Daurès M, John M, Grangeon JP, Gourinat AC. Co-infection with Zika and Dengue Viruses in 2 Patients, New Caledonia, 2014. Emerg Infect Dis. 2015. 21(2):381-2.*
- *Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. Emerg Infect Dis. 2015. 21(1):84-6.*
- *Faye O, Freire CCM, Iamarino A, Faye O, de Oliveira JVC, Diallo D, Zanotto PM, Sall AA. Molecular Evolution of Zika Virus during Its Emergence in the 20th Century. Plos Neglect Trop Dis. 2014: 8 (1): e2636. doi:10.1371/journal.pntd.0002636*
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- *Faye O, Faye O, Dupressoir A, Weidmann M, Ndiaye M, Sall. One-step RT-PCR for detection of Zika virus, J Clin Virol, 2008: 43, 96-101*

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.

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

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

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.

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

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

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.

> Gastric cancer is the fourth most common malignancy and the third cause of cancer related-death worldwide.

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.

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

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

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 n° 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.

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

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.

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).

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.

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

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

Drug discovery to treat the neglected area of trypanosomatids

Project ACIP n° 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.

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

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.

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

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.

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

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

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.

Hemorrhagic fevers caused by DENV and YFV can be life-threatening when left untreated.

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.

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

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.

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

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).

Dengue virus infection is a serious health problem. 2.5 billion people wordlwide are exposed to dengue virus.

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.

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

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.

No specific treatment exists to defeat dengue or chikungunya.

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

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

Project ACIP n° 01-2016

Coordinator: Dr Anubis VEGA RUA, Laboratory of Medical Entomology – Institut Pasteur in Guadeloupe
Collaborators from the Institut Pasteur International Network: Dr Isabelle DUSFOUR, Institut Pasteur in French Guiana, Drs Claude GUERTIN and Philippe CONSTANT, INRS – Institut Armand Frappier (Canada) *

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.

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

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.

In April 2015, more than 1,379,788 chikungunya suspected cases had been recorded in the Caribbean and American continent.

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).*

Better understanding dengue virus evolution to better protect populations

Project ACIP n° 06-2016

Coordinator: Dr Myrielle DUPONT-ROUZEYROL, Dengue and Arboviruses Expertise and Research Unit – Institut Pasteur in New Caledonia
Collaborators from the Institut Pasteur International Network: Dr Veasna DUONG, Institut Pasteur in Cambodia, and Dr Louis LAMBRECHTS, Institut Pasteur (Paris) *

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.

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

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.

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

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).*

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

Project ACIP n° 22-2016

Coordinator: Dr Abdou Kader NDIAYE – Enteric Viruses Laboratory - Institut Pasteur in Dakar
Collaborators from the Institut Pasteur International Network: Dr Ionela GOUANDJIKA, Institut Pasteur in Bangui, Dr Francis DELPEYROUX, Institut Pasteur (Paris)

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).

Strengthening hygiene is important in preventing.

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.

Neurological consequences and even death are possible in the infant.

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.

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

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.

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

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.

> Diabetes prevalence has been rising more rapidly in middle- and low-income countries.

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.

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

THE PROJECT

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 directky caused by diabetes.

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

Project ACIP n°57-2017

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.

Obesity and type 2 diabetes concern 900 million individuals around the world.

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.

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

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.

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

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).*

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.

30–50% of the global population has been exposed to and may be chronically infected with T. gondii.

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.

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

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.

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

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.

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 quinoline 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 anti-malarial 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 .

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

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.

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.

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

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.

> 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 also involves / involved the participation of external partner(s).*

THE PROJECT

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 virus-mosquito-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 is endemic in more than 120 countries around the world.

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.

Over half of the global population is at risk for 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.

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

THE PROJECT

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 capabilty 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.

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

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

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).*

THE PROJECT

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.

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

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

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

In most cases, vector control is the only method at hand. Many emerging or re-emerging 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.

THE PROJECT

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.

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

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.

> 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.

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

THE PROJECT

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 (arthropod-borne 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.

> WHO estimates that 3.9 billion people, in 128 countries, are at risk of infection with dengue virus.

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.

> Approximately 390 million dengue infections each year worldwide.

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.

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

THE PROJECT

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.

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

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.

> 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.

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.

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

THE PROJECT

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

Scientific coordinator: Dr Milena LESEVA, Laboratory of Experimental Immunotherapy - Stephan Angeloff Institute (Bulgaria)

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.

> 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.

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?

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

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.

THE PROJECT

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

Project ACIP n° 358-2020

Scientific coordinator: Dr Teca GALVAO, Laboratório de Genômica Funcional e Bioinformática - Fiocruz (Brazil)
Institut Pasteur International Network collaborators: Dr Ana Paula CARVALHO ASSEF, Fiocruz, Dr Ariel MECHALY (Crystallography Platform) and Dr Remy LE MEUR (Biological NMR Technological Platform), Institut Pasteur (Paris) and Alejandro BUSCHIAZZO, Institut Pasteur in Montevideo *

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.

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

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.

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

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.

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

THE PROJECT

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.

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

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.

THE PROJECT

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

Scientific coordinator: Dr Muriel VRAY, Emerging Diseases Epidemiology Unit - Institut Pasteur (Paris)
Institut Pasteur International Network collaborators: Dr Claude FLAMAND, Institut Pasteur in French Guiana, Dr Christian MALAKA, Institut Pasteur in Bangui *

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 person-to-person as a result of poor hygiene.

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

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.

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

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.

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

THE PROJECT

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

Scientific coordinators: Dr Arnaud TARANTOLA and Dr Perrine PARIZE, Lyssavirus, Epidemiology and Neuropathology Unit - Institut Pasteur (Paris)
Institut Pasteur International Network collaborators: Dr Sowath LY, Dr Tineke CANTAERT and Dr Veasna DUONG, Institut Pasteur in Cambodia, Dr Rindra RANDREMANANA and Dr Philippe DUSSART, Institut Pasteur de Madagascar and Dr Hervé BOURHY, Institut Pasteur (Paris)

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.

> 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.

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.

> 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.

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”.

THE PROJECT

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.

PORTFOLIO TRANSVERSAL RESEARCH PROGRAMS (PTR) FUNDED FROM 2014 TO 2020

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.

> Usher syndrome affects one in 30,000 people in France.

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.

> Usher syndrome is responsible for the majority of deafblindness.

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.

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.

> Worldwide, EV-A71 is a common cause of hand, foot, and mouth disease in infants and young children.

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.

> Some people infected with enteroviruses have no symptoms but still can spread the virus to others.

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.

** 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

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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.

More than one billion people are now exposed to *Plasmodium vivax*.

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 *sporozoïte* 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.

About 90 million cases of malaria are caused by P. vivax worldwide each year.

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 *merozoïte* 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.

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.

> 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 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.

> 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?

*Helicobacte*r is a genus of Gram-negative bacteria belonging to the Epsilon-proteobacteria 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.

> *Hp* infects the stomach of up to 50% of the human population worldwide.

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?

> 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.

> More than 12 million humans are currently infected in the world.

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.

> *Leishmania* parasite causes 1,3 million new cases and the death of 40,000 people per year.

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.

> Populations at risk of visceral leishmaniasis are children from 1 to 3 years

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.

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 anti-leishmanial 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.

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.

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.

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.

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.

An estimated 150,000 to 200,000 cases of HFRS occur each year worldwide.
Depending on the virus, the death rate ranges from <1% to >12%.

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 hantavirus life cycle. Another goal will be to produce recombinant antigens of 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

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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.

For the first time, virulence characteristics of *Bacillus cereus* strains could be predicted.

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).

Identification of biomarkers to be targeted in link with the pathogenicity and species differentiation within the *Bacillus cereus* group.

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.

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 BioImaging), 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.

Listeria is responsible for the deadliest bacterial foodborne outbreak in the US (California, 1985).

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.

In 2010, *Listeria* infected 23,150 persons worldwide and killed 5,463 (highest mortality rate among foodborne pathogens).

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.

Biologists use *Listeria* as a molecular model to manipulate cells & to study human biology.

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

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.

Alzheimer's and related diseases affect over 900,000 people in France and over 35 million in the world in 2010.

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 (α-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 α-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 » α-syn in Lewy bodies between neurons, similar to what occurs in prion diseases. The transmission mechanisms of α-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.

In the world, about 5 million people suffer from Parkinson's disease and 300,000 individuals are diagnosed each year.

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 α-syn transmission in human neurons. Working together, the three groups will be in a unique position to characterize the mechanism of α-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.

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

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.

900 million people in the world are exposed to the risk of contracting the virus.

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.

Every year in the world, 200,000 cases of yellow fever among which 30,000 are mortal.

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.

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.

Aspergillus fumigatus are involved in 80% to 90% of human Aspergillosis.

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.

Aspergillus fumigatus is responsible for 200,000 deaths per year worldwide.

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 rodlet-based 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.

The mortality rates of invasive Aspergillosis range from 40%-90% in risk groups.

Decipher *Plasmodium falciparum* mechanism of resistance to Artemisinin

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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.

> Resistance to ACT represents a major threat to public health.

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.

> Resistance to ACT will impact the global strategy to fight this disease.

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*.

> 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.

Every year in the world, there were 1,3 million new cases of Leishmaniasis .

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).

Each year 500,000 patients contract the most serious form of the disease.

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.

Leishmania parasite causes the death of 20,000 to 30,000 people per year in the world.

The microbiota: the forgotten organ!

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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.

> The composition of gut microbiota is unique to each individual, just like our fingerprints !

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).

> *Escherichia coli* is a bacterium that can be found in human intestines.

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 PROJECT

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 mono- or 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 bacteria-bacteria 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 in *Anopheles* mosquitoes on their capacity to 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.

Sleeping sickness threats the populations of 36 sub-Saharan countries.

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.

65 million individuals are exposed to *Trypanosoma* and there are 20,000 estimated cases.

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 co-infections, 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.

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.

Worldwide, influenza outbreaks are responsible for 3 to 5 million cases of severe illnesses of which 250.000 to 300.000 lead to death.

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.

In France, 2 to 7 million people are affected by influenza, every winter.

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 essential for viral replication in human cells, to try to block these interactions for preventive or therapeutic purposes.

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

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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.

Antibiotic resistance is responsible for 700,000 deaths per year worldwide.

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.

In 2050, antibiotic resistance could become a top cause of death outpacing cancer.

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.

In the world, 200 newborns die per hour following a bacterial infection.

Understanding the relationship between malnutrition / Pediatric Environmental Enteropathy 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.

According to WHO, the malnutrition is responsible for more than 3 millions deaths per year in children under age 5 years.

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.

In the world, one child in four aged under 5 suffer from chronic malnutrition.

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.

In Madagascar, 47% of children under 5 years are affected by chronic malnutrition.

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

Better understanding host/virus interactions in order to develop therapeutic strategies

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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.

An estimated 440000-1300000 Zika virus infections have occurred in the most outbreak in Brazil.

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."

Zika virus has been reported in more than 70 countries and territories worldwide since 2015.

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.

There is no cure and no vaccine for Zika, Chikungunya diseases.

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

Better understanding bacteria-host interactions in allergic asthma to prevent it and treat it

PTR | INSTITUT PASTEUR

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.

235 million people suffer from asthma in the world. It is the most common chronic disease among children.

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.

In France, asthma affects more than 4 million people, one third of whom are under the age of 15.

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.

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.

In the world, 2 billion people have been infected by hepatitis B virus and over 370 million are chronic carriers.

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?

In 2015, hepatitis B resulted in 887,000 deaths (including cirrhosis and hepatocellular carcinoma).

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.

In France, over 3 million people have been a contact with HBV, 300,000 are chronic carriers.

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

Better understanding of the invasive capabilities of bacteria in order to develop new drugs and assist in diagnosis

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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.

Shigellosis kills from 700,000 to 1 million people each year in the world.

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.

In 2015, 10,4 million people fell ill with **TB** and 1,8 million died from the disease.

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**.

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

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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.

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.

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?

More than one million people are estimated to die from *Shigella* infection each year.

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.

Some 580,000 cases of Shigellosis are reported among travellers and military personnel from industrialized countries.

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

Study Aspergillosis in Cambodia to investigate the impact of this pathogen in East Asia

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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.

> Over 300 million people are acutely or chronically infected by fungi, leading to death, long term illness, blindness, reduced work capacity.

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 major chronic, invasive and allergic forms of aspergillosis account for around 600,000 deaths annually worldwide.

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.

> 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 best-known 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.

> Many bacteria of the human microbiome are still poorly studied.

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 *Negativicutes* may have a dual role, beneficial and pathogenic.

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.

> 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.

THE PROJECT

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.

Pseudomonas aeruginosa, third cause of nosocomial infections in France.

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.

In the patients with cystic fibrosis, the bacteria contribute to the decline of the respiratory function.

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.

About 60% of people with cystic fibrosis have a chronic respiratory infection caused by *P. aeruginosa*.

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

Better knowledge of nAChRs and identification of new ligands for therapeutic purposes

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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.

Schizophrenia, mental disorder, that affects more than 21 million people in the world.

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.

Alzheimer's disease in France: 900,000 patients, 225,000 new cases diagnosed per year.

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.

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 HP1α, HP1β and HP1 γ 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.

Epigenetic regulator of cellular ageing.

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.

Better understanding of genetic epidemiology and pathogen evolution of *Leptospira* will improve diagnostic and prevention

Project PTR n° 30-2017

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.

> Leptospirosis is a zoonotic and emerging bacterial disease.

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.

> Leptospirosis cause more than 1 million severe cases with 60,000 deaths per year.

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.

> In Europe, the highest incidence is observed in France (600 cases per year).

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

Development of a new genetration of anti-tuberculosis 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 is still among the 10 top causes of death worlwide.

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.

> It is estimated that about a third of the world's population are infected with *Mycobacterium tuberculosis*.

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.

THE PROJECT

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.

How *leptospires* escape from phagocytes?

Humans develop a mild to severe acute disease potentially fatal, due to multi-organ 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.

Use TLR and NOD agonists to restore phagocyte functions?

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).*

A comprehensive understanding on the ni-transport 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.

Helicobacter pylori infects about 50% of the world's population.

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.

H. pylori is the most common cause of gastric ulcers and gastritis.

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.

H. pylori is the main cause of gastric cancers.

THE PROJECT

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

PTR | INSTITUT PASTEUR

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.

Malnutrition causes the death of 3 to 5 million children under 5 every year in the world.

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.

Childhood stunting affects 162 million children under the age of 5 years in the world

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 will offer new solutions to prevent and treat the deleterious effects of PEE

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).*

Develop rapidly tools for diagnostics of emerging diseases through the use of new technologies (nanobodies, droplet based microfluidics)

PTR | INSTITUT PASTEUR

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.

> The annual global cost of moderately severe to severe pandemics is roughly $570 billion, or 0.7% of global.

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.

> Rapid development of tools for diagnostics.

The nanobodies (or VHHs) are antibodies devoid of light chains presenting antigen-binding 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-of-concept 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.

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.

Incidence of CS about 1/200000 in European countries.

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.

No prevention strategy or known cure for CS.

It is therefore essential to understand molecular mechanisms involved in this premature ageing pathology.

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

THE PROJECT

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 inflammatory bowel disease, which includes Crohn's disease and ulcerative colitis.

Colorectal cancer is a global threat with more the 1 million of new cases and more than 700,000 deaths each year.

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.

Colerectal cancer is the fourth cause of tumor death worldwide.

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.

Colorectal cancer is the second most common cancer in women and third in men.

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.

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

THE PROJECT

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.

> 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.

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.

> 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.

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.

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

THE PROJECT

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 BioImaging), 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.

> Worldwide, it is estimated that approximately 15 million pertussis cases and about 200 000 pertussis deaths occur per year.

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.

> The CyaA toxin constitutes a potent non-replicating vector to deliver antigens into antigen presenting cells and induce specific cell mediated immune responses.

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.

THE PROJECT

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.

> Although over 57 million people are currently at risk of contracting HAT in rural sub-Saharan Africa.

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 number of cases reported in 2017 has dropped to approximately 1,442 from only a dozen African countries.

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.

> 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.

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

Validation of a next generation diagnostic assay for antimicrobial resistance detection in order to enhance personalized treatment of MDR-TB patients

Project PTR n° 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.

> 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.

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)).

> 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.

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

THE PROJECT

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.

> 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.

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.

> Chagas Disease: approximately 6-7 million people infected, with 70 million at risk.

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 PROJECT

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.

Half of the world's population live in areas where the mosquito and the virus are circulating.

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.

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.

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.

Dengue is increasing at a higher rate than most other communicable diseases, with a doubling in incidence every 10 years since 1990

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

THE PROJECT

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 first case of human infection with monkeypox was described in 1970 in the Democratic Republic of the Congo.

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.

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.

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.

In 2019, monkeypox imported cases from Nigeria are reported In United Kingdom, Singapore and Israel.

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 PROJECT

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.

Amoebiasis is the third deadliest parasitic disease in the world.

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.

Shigellosis kills hundreds of thousands of people around the world every year, especially children under the age of 5.

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

THE PROJECT

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.

Lack of knowledge of the impact of mechanical stresses on tissue invasive process.

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.

Need to develop non invasive methods to quantify physical parameters of tissue architecture.

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.

Identify new active compounds against multidrug-resistant 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.

> It is a global health problem, still causing more than 450.000 deaths each year (mostly African children) and over 200 million infections.

Treatments are available to kill the parasite, but *Plasmodium* is becoming more and more resistant to these therapies.

> 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.

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.

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

THE PROJECT

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

Scientific coordinator: Dr Laurent DACHEUX, Lyssavirus Epidemiology and Neuropathology Unit - Institut Pasteur (Paris)
Institut Pasteur International Network collaborators: Dr Marcel HOLLENSTEIN, Institut Pasteur (Paris), Dr Philippe DUSSART and Dr Véronique CHEVALIER, Institut Pasteur in Cambodia, Dr Soa Fy ANDRIAMANDIMBY and Dr Hélène GUIS, Institut Pasteur in Madagascar*

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.

> This viral disease is still responsible for an estimated 60,000 human deaths per year, due to dog-mediated rabies for 99% of cases.

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.

> 40% of people bitten by suspect rabid animals are children under 15 years of age.

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

THE PROJECT

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

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Project PTR n° 245-2019

Scientific coordinator: Dr Zeynep BAHAROGLU, Baterial Genome Plasticity Unit - Institut Pasteur (Paris)
Institut Pasteur (Paris) collaborators : Dr Frédéric BARRAS, Dr Eduardo ROCHA *

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.

> 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.

AMINOGLYCOSIDE CLASS OF ANTIBIOTICS

Aminoglycosides (AGs) are antibiocs known for their high efficiency against Gram-negative 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 can affect anyone, of any age, in any country.

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

THE PROJECT

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 γ - 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

Scientific coordinator: Dr Raphaël ETOURNAY, Genetics and Physiology of Hearing Unit - Institut Pasteur (Paris)
Institut Pasteur International Network collaborators : Dr Samy GOBAA, (Biomaterials & Microfluidics Core Facility) Institut Pasteur (Paris), Dr Régis GRAILHE, Institut Pasteur in Korea *

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.

> Hearing loss affects more than 460 million people worldwide.

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.

> It is estimated that by 2050 over 900 million people will have disabling hearing loss.

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

THE PROJECT

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

Scientific coordinator: Dr Barbara GAYRAUD-MOREL, Stem Cells and Development Unit - Institut Pasteur (Paris)
Institut Pasteur International Network collaborators : Dr Roberto BRUZZONE, Hong Kong University - Pasteur Research Pole, Dr Fabrice CHRETIEN, Institut Pasteur (Paris) *

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 .

> Each year about 3-5 million individuals develop a severe flu, causing 290 000 to 600 000 deaths.

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

> Children and elderly patients are at higher risk of skeletal muscle-flu related complications.

* *This project also involves the participation of external partner.*

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 influenza-related 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).

> Severe bacterial infections are a leading cause of death in children under 5 in low income countries (LICs) with neonates bearing the highest burden.

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).

> It has been estimated that almost 7 million cases of neonatal infections possibly occurred each year.

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

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

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

Scientific coordinator: Dr Nadia BENAROUDJ, Biology of Spirochetes Unit - Institut Pasteur (Paris)
Institut Pasteur International Network collaborators: Dr Rosario DURAN, Institut Pasteur in Montevideo, Dr Catherine WERTS, Institut Pasteur (Paris) *

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.

> More than one million cases of leptospirosis are currently reported annually in the word, with 10% of mortality.

The manifestations of this infection range from flu-like symptoms to a severe life-threatening 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 witihin *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.

> 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 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*.

* *This project also involves / involved the participation of external partner.*

THE PROJECT

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

Scientific coordinator: Dr Eliette TOUATI, Unit of Helicobacter Pathogenesis - Institut Pasteur (Paris)
Institut Pasteur International Network collaborators: Dr Amel METTOUCHI and Dr Paola B. ARIMONDO, Institut Pasteur (Paris), Dr Dionyssios N. SGOURAS, Hellenic Pasteur Institute (Athens, Greece)

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.

Colorectal cancer is a global threat with more than 1.8 million of new cases and more than 800,000 deaths worldwide each year.

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 already the second leading cause of cancer death in the world, and its incidence is steadily rising in developing nations.

THE PROJECT

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 pro-tumorigenic 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

Scientific coordinator: Dr Rogerio AMINO, Malaria Infection and Immunity Unit - Institut Pasteur (Paris)
Institut Pasteur International Network collaborators: Dr Pierre CHARNEAU and Dr Sylvie GARCIA, Institut Pasteur (Paris) and Dr Helder NAKAYA, Scientific Platform Pasteur - University of Sao Paulo (Brazil)

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.

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.

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.

Plasmodium falciparum is responsible for ~97% of malaria cases being the most important human- infecting plasmodial species.

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.

THE PROJECT

Four teams from two institutes of Institut Pasteur International Network (Paris and UPS- Sao Paulo) will combine their scientific expertise (on plasmodial pre-erythrocytic 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.

Retroviruses are very common in the animal world.

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 ?

Retroviruses crossed the barrier species on multiple occasions.

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.

THE PROJECT

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

Scientific coordinator: Dr Marcos COSTA, Molecular determinants of neurodegenerative diseases Laboratory - Institut Pasteur in Lille (France)
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-β peptides (amyloid plaques) and intracellular inclusions of neurofibrillary tangles rich in microtubule-associated protein tau are pathological hallmarks of the disease.

> 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.

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.

> Dementia is one of the major causes of disability and dependency among older people worldwide.

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.

THE PROJECT

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-β 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 anti-campylobacteriosis vaccine relying on chemical synthesis

Project PTR n° 380-2020

Scientific coordinator: Dr Charles GAUTHIER, Centre Armand Frappier Santé Biotechnologie, INRS (Canada)
Institut Pasteur International Network collaborators: Dr Laurence MULARD, Institut Pasteur (Paris) and Dr Charles DOZOIS, Centre Armand Frappier Santé Biotechnologie, INRS (Canada)

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.

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.

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 infections are generally mild, but can be fatal among very young children, elderly, and immunosuppressed individuals.

THE PROJECT

Capitalizing on their strong background in the field of synthetic carbohydrate-based 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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Institut Pasteur (Paris) collaborators: Dr Fabrice DE CHAUMONT and Dr Florent HAISS *

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.

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.

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

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.

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

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 cognition-related 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

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Project PTR n° 395-2020

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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.

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*.

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.

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.

Notes

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