**PhD PROPOSAL FOR THE**

**PASTEUR - PARIS UNIVERSITY INTERNATIONAL DOCTORAL PROGRAM**

Time for applicants to contact host laboratories: September 13 – November 2, 2017

Deadline for full application: November 13, 2017

Interviews: January 30, February 2, 2018

Start of the Ph.D.: October 1, 2018

**Title of the PhD project:** Investigation of the role of neuronal, microglial and glial cells during rabies virus infection in the modulation of the innate immune response

**Keywords:** Brain, Rabies, inflammation, neurons, glial cells, microglial cells

**Department:** Infection and Epidemiology

**Name of the lab:** Unit Lyssavirus Dynamics and Host Adaptation (DyLAH)

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***Doctoral school affiliation and University*:** Bio SPC

Presentation of the laboratory and its research topics:

DyLAH unit hosts the National reference centre and the WHO collaborative center for rabies.

The overall strategy of the Unit DyLAH is to address innovative and pertinent scientific questions related to rabies and other related infectious diseases that target the central nervous system. In response to the complexity of the mechanisms involved and of the questions addressed, this is achieved through the integration of many different approaches and competences present in the laboratory and developed through collaborations with relevant partners in other research laboratories located in France and abroad (mainly Belgium, United Kingdom, Germany, Spain, Korea, China, Cambodia and Australia).

Specifically, research activities of the Unit DyLAH focus towards the acquisition and diffusion of new, basic knowledge regarding (i) the evolution of lyssaviruses, (ii) the biology of lyssavirus infection, particularly the determinants and processes involved in their virulence factors and host adaptation, (iii) the host response and the pathologic processes, and (iv) the drivers of the dynamics of lyssavirus spread in natural populations of susceptible animals. The acquired knowledge is then translated into strategies and tools for the control of rabies in the field.

Unit DyLAH particularly aims at supporting research projects on lyssaviruses, rabies and related subjects for which it has a distinctive and well recognize expertise. However, in addition it has always been keen to engage in new biological or epidemiological challenges on related questions or broader fundamental questions (i.e. the global understanding of the process of emergence: PREDEMICS program; the understanding of the viral replication complex and the development of antiviral molecules of large spectrum: FP7/SILVER, ANR RAB-CAP; etc.).

To achieve its scientific objectives, the Unit DyLAH has taken advantage of its resources, infrastructure and location in the campus of Institut Pasteur. This situation creates a unique and fruitful interface between its researchers, the other colleagues from the Department Infection and Epidemiology and the rest of the departments, technical platforms and facilities located on the campus. Further, and notably via the International network of Institut Pasteur and its relation with WHO, the Unit DyLAH has developed long lasting and close relationships with several institutions and colleagues located in enzootic countries and therefore benefits from direct interaction with the field, establishing a continuum of activities between fundamental research, development and field applications.

This provides an optimized setting for the rapid identification of relevant scientific and public health questions, their rapid integration as research questions and their study in a fundamental and integrated manner to provide science driven and meaningful solutions when needed.

Description of the project:

Rabies is an untreatable disease of humans, which has a case-fatality rate of almost 100% in non-vaccinated individuals. The etiological agents of rabies are viruses of the globally distributed *Lyssavirus* genus, the best characterized of which is rabies virus (RABV) that infects diverse mammalian species with transmission to humans most commonly through bites from infected dogs [1]. RABV is a strictly neurotropic virus. After entry in the peripheral nervous system, the virus spreads within axons by retrograde fast axonal transport, then disseminates throughout the central nervous system along neuroanatomical connections [2, 3] and finally spreads to multiple organs along autonomic and/or sensory nerves. Successful achievement of the virus cycle relies on the preservation of the neuronal network [4]. The surprising lack of major histopathological changes and neuronal death raised the hypothesis that the clinical outcomes resulted from major neuronal dysfunction, the basis of which remained so far unknown. Further, the contribution of glial and microglial cells and their crosstalk with neuronal cells remained undetermined [5].

In the recent years, it has become clear that the host innate immune system and the viral countermeasures profoundly shape this RABV-host relationship. RABV seems to minimize the inflammation in the nervous tissues it infects [6, 7]. The evasion of host innate immunity by pathogenic lyssaviruses depends on a unique mechanism of selective targeting of interferon-activated STAT proteins by the phosphoprotein (P protein) [8-12]. The matrix (M) protein also plays some pivotal roles during the viral life cycle [14]. It modulates the balance between replication and transcription of the viral genome and induces neuronal cell death [15, 16]. We recently described and characterized a new splicing variant of the NF-κB family, RelAp43, targeted by the M protein of field isolates of rabies virus [17]. This transcription factor is able to modify the equilibrium between the different NF-κB dimers. The death or survival of RABV-infected neurons is also controlled by the G protein via a PDZ binding site [18].

As most of the works trying to decipher the regulation of the innate immune pathways and of inflammation during RABV-infection were performed in laboratory cell lines. this project is aiming at studying this question in a more physiological context. The global objective of this project is to study *in vivo* and *in vitro* the role of neuronal, microglial and glial cells taken as separate cell compartments and taken as a whole to understand their respective and complementary roles in the modulation of innate immune response during RABV infection. Furthermore, the respective effects of M and P proteins on innate immunity and inflammation will be further investigated *in vitro* and *in vivo* using relevant recombinant RABV in order to identify the cellular and the viral molecular factors involved in the virulence of RABV.

More specific objectives are to determine:

* Susceptibility and damage to neuronal, microglial and astroglial cells during RABV infection (**Infection, inflammation, cell activation, apoptosis and death**)
* Role of the different cellular compartments of the brain in the innate immune response to RABV infection (**brain structure and cell type**)
* Role of the virus proteins in the modulation of the innate immune response in the different cellular compartments of the brain (**use of recombinant RABV**)
* Respective contributions of the cell signalling pathways in the response of the different cellular compartments of the brain (**transcriptomic, RNA seq, siRNA)**

This project should generate cutting-edge technological developments and knowledge of how RABV and brain interact to maintain health or drive pathology. The resulting knowledge will offer new opportunities to monitor rabies-infected patients and save their life.

References:

1. Fooks AR, Banyard AC, Horton DL, Johnson N, McElhinney LM, Jackson AC. Current status of rabies and prospects for elimination. Lancet. 2014;384(9951):1389-99. doi: 10.1016/S0140-6736(13)62707-5. PubMed PMID: 24828901.

2. Ceccaldi PE, Ermine A, Tsiang H. Continuous delivery of colchicine in the rat brain with osmotic pumps for inhibition of rabies virus transport. Journal of virological methods. 1990;28(1):79-83. PubMed PMID: 1693370.

3. Ugolini G. Rabies virus as a transneuronal tracer of neuronal connections. Advances in virus research. 2011;79:165-202. doi: 10.1016/B978-0-12-387040-7.00010-X. PubMed PMID: 21601048.

4. Jackson AC. Rabies. In: Jackson AC, editor. Viral Infections of the Human Nervous System: Springer Basel; 2013. p. 211-35.

5. Jackson AC, Phelan CC, Rossiter JP. Infection of Bergmann glia in the cerebellum of a skunk experimentally infected with street rabies virus. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire. 2000;64(4):226-8. PubMed PMID: 11041500; PubMed Central PMCID: PMC1189622.

6. Baloul L, Lafon M. Apoptosis and rabies virus neuroinvasion. Biochimie. 2003;85(8):777-88. PubMed PMID: 14585545.

7. Hicks DJ, Nunez A, Healy DM, Brookes SM, Johnson N, Fooks AR. Comparative pathological study of the murine brain after experimental infection with classical rabies virus and European bat lyssaviruses. Journal of comparative pathology. 2009;140(2-3):113-26. doi: 10.1016/j.jcpa.2008.09.001. PubMed PMID: 19111840.

8. Brzozka K, Finke S, Conzelmann KK. Identification of the rabies virus alpha/beta interferon antagonist: phosphoprotein P interferes with phosphorylation of interferon regulatory factor 3. Journal of virology. 2005;79(12):7673-81. doi: 10.1128/JVI.79.12.7673-7681.2005. PubMed PMID: 15919920; PubMed Central PMCID: PMC1143667.

9. Brzozka K, Finke S, Conzelmann KK. Inhibition of interferon signaling by rabies virus phosphoprotein P: activation-dependent binding of STAT1 and STAT2. Journal of virology. 2006;80(6):2675-83. doi: 10.1128/JVI.80.6.2675-2683.2006. PubMed PMID: 16501077; PubMed Central PMCID: PMC1395475.

10. Wiltzer L, Larrous F, Oksayan S, Ito N, Marsh GA, Wang LF, et al. Conservation of a unique mechanism of immune evasion across the Lyssavirus genus. Journal of virology. 2012;86(18):10194-9. doi: 10.1128/JVI.01249-12. PubMed PMID: 22740405; PubMed Central PMCID: PMC3446585.

11. Wiltzer L, Okada K, Yamaoka S, Larrous F, Kuusisto HV, Sugiyama M, et al. Interaction of rabies virus P-protein with STAT proteins is critical to lethal rabies disease. The Journal of infectious diseases. 2014;209(11):1744-53. doi: 10.1093/infdis/jit829. PubMed PMID: 24367042.

12. Assenberg R, Delmas O, Ren J, Vidalain PO, Verma A, Larrous F, et al. Structure of the nucleoprotein binding domain of Mokola virus phosphoprotein. Journal of virology. 2010;84(2):1089-96. doi: 10.1128/JVI.01520-09. PubMed PMID: 19906936; PubMed Central PMCID: PMC2798355.

13. Fouquet B, Nikolic J, Larrous F, Bourhy H, Wirblich C, Lagaudriere-Gesbert C, et al. Focal adhesion kinase is involved in rabies virus infection through its interaction with viral phosphoprotein P. Journal of virology. 2015;89(3):1640-51. doi: 10.1128/JVI.02602-14. PubMed PMID: 25410852; PubMed Central PMCID: PMC4300764.

14. Graham SC, Assenberg R, Delmas O, Verma A, Gholami A, Talbi C, et al. Rhabdovirus matrix protein structures reveal a novel mode of self-association. PLoS pathogens. 2008;4(12):e1000251. doi: 10.1371/journal.ppat.1000251. PubMed PMID: 19112510; PubMed Central PMCID: PMC2603668.

15. Gholami A, Kassis R, Real E, Delmas O, Guadagnini S, Larrous F, et al. Mitochondrial dysfunction in lyssavirus-induced apoptosis. Journal of virology. 2008;82(10):4774-84. doi: 10.1128/JVI.02651-07. PubMed PMID: 18321977; PubMed Central PMCID: PMC2346764.

16. Larrous F, Gholami A, Mouhamad S, Estaquier J, Bourhy H. Two overlapping domains of a lyssavirus matrix protein that acts on different cell death pathways. Journal of virology. 2010;84(19):9897-906. doi: 10.1128/JVI.00761-10. PubMed PMID: 20631119; PubMed Central PMCID: PMC2937802.

17. Luco S, Delmas O, Vidalain PO, Tangy F, Weil R, Bourhy H. RelAp43, a member of the NF-kappaB family involved in innate immune response against Lyssavirus infection. PLoS pathogens. 2012;8(12):e1003060. doi: 10.1371/journal.ppat.1003060. PubMed PMID: 23271966; PubMed Central PMCID: PMC3521698.

18. Prehaud C, Wolff N, Terrien E, Lafage M, Megret F, Babault N, et al. Attenuation of rabies virulence: takeover by the cytoplasmic domain of its envelope protein. Science signaling. 2010;3(105):ra5. doi: 10.1126/scisignal.2000510. PubMed PMID: 20086240.

Expected profile of the candidate (optional):

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