Internship Proposal

Project's title: HSV1 primary infection kinetics and immunity on the risk of further reactivation.

Supervisor's name: Marc Labetoulle

Welcoming institution and team (short presentation in 10 lines maximum)

The Department of "Infectious Disease Models for Innovative Therapies" (IDMIT, Head: Roger Le Grand) brings together more than 100 scientists, PhDs or MD-PhDs, working on common scientific challenges related to innate and adaptive immunity in the context of autoimmune and infectious disorders. IDMIT benefits from outstanding facilities, advanced technologies for *in vivo* imaging, pharmacology, and the assessment of immune responses and treatments. The supervisor of the project (Marc Labetoulle) is specialised in ocular infectious diseases and more specifically in herpetic eye disease, and already coordinates large cohorts of patients and therapeutic programs, together with experimental *in vivo* projects focused on the study of latency and reactivation of Herpes simplex virus, based on a mouse model developed in his team. In-house imaging and flow cytometry platforms will be utilised for this project.

IDOH co-supervisor: Antoine Rousseau & Oscar Haigh

Summary of the project (1page max):

Herpes simplex virus type 1 (HSV1) infection is responsible for ocular pathologies, of which keratitis is the most common. Recurrent and severe keratitis constitute the primary cause of low vision from infectious origin in Western countries. In humans, the virus is acquired during a usually asymptomatic oral primary infection, after which resides in a state of latency in both trigeminal ganglia (TG), the heart of sensory innervation of the face. While nearly 100% of individuals harbour HSV1 in a latent form in both TG, clinical recurrences caused by viral reactivation are almost always unilateral. This phenomenon, that is still not understood, suggests that a regulatory mechanism permanently forces the virus to remain quiescent in one of the 2 TG; with no possibility of reactivation. The understanding of this phenomenon could pave the way for new preventive therapeutic strategies that are essential, considering that available treatments against HSV1 only reduce duration and severity of clinical episodes; they are not prophylactic. Furthermore, vaccine strategies are yet to provide effective protection in humans.
Animal experiments are necessary to study, not only the herpetic ocular manifestations, but especially the phenomena of alternating latency and reactivation. Our team has developed a mouse oro-ocular (OO) model that faithfully reproduces infection in humans. In this model, labial inoculation (reproduction of the primary oral infection in humans), is followed by an

acute infection accompanied by ocular pathology, then entry of the virus into latency occurs in connected neurological structures, particularly in TG.

Our recent experiments using the OO model demonstrated that a primary infection in the right lip by a live, but non-neurovirulent HSV1 (primary infective strain), can constrain a fully virulent wild-type strain inoculated a few days later in the left lip (secondary infective strain). A latent infection was established by this technique without any reactivation capacity. Further studies are imperative to better understand the mechanisms underlying these new results.

This project aims to combine innovative immuno-virological and imaging techniques to understand the dynamic phenomena of innate and adaptive immunity involved, and define the interrelationship with kinetics of infection by the putative vaccine strain. Initially, effectors of immunity (innate and adaptive) will be studied to define immune responses associated with the TG. Primary culture of these tissues subjected to HSV1 infection will also be studied by immunohistochemistry, molecular biology, and flow cytometry techniques. Secondly, modified viruses expressing fluorescent proteins will be exploited to determine the state of viral replication in time and space by the primary-infective strain, then by the second strain.

These studies will provide a global visualisation of the TG network and associated immunity that develops in our model; furthering our understanding of unilateral activation phenomenon and open perspective toward the development of immunisation strategies.

Supervisor's Significant publications (5 maximum)

1) Recurrent herpetic keratitis despite antiviral prophylaxis: A virological and pharmacological study.
Rousseau A, Boutolleau D, Titier K, Bourcier T, Chiquet C, Weber M, Colin J, Gueudry J, M'Garrech M, Bodaghi B, Burrel S, Agut H, Deback C; HEDGOF (Herpes Eye Disease Group of France), **Labetoulle M.**
Antiviral Res. 2017 Oct;146:205-212.

2) Persistent Impairment of Quality of Life in Patients with Herpes Simplex Keratitis.
Reynaud C, Rousseau A, Kaswin G, M'garrech M, Barreau E, **Labetoulle M.**
Ophthalmology. 2017 Feb;124(2):160-169.

3) Latency Entry of Herpes Simplex Virus 1 Is Determined by the Interaction of Its Genome with the Nuclear Environment.
Maroui MA, Callé A, Cohen C, Streichenberger N, Texier P, Takissian J, Rousseau A, Poccardi N, Welsch J, Corpet A, Schaeffer L, **Labetoulle M**, Lomonte P.
PLoS Pathog. 2016 Sep 12;12(9):e1005834.

4) Biological features of herpes simplex virus type 1 latency in mice according to experimental conditions and type of neurones.
Cavallero S, Huot N, Francelle L, Lomonte P, Naas T, **Labetoulle M.**
Invest Ophthalmol Vis Sci. 2014 Oct 16;55(12):7761-74

5) HSV-1 genome subnuclear positioning and associations with host-cell PML-NBs and centromeres regulate LAT locus transcription during latency in neurons.
Catez F, Picard C, Held K, Gross S, Rousseau A, Theil D, Sawtell N, **Labetoulle M**, Lomonte P.
PLoS Pathog. 2012;8(8).