

In vivo Imaging of the HIV/SIV - Host Interactions

L'Unité mixte de recherche UMR1184/ImVA-HB (Center for Immunology of Viral, Auto-immune, Hematological and Bacterial diseases) et le département IDMIT (Infectious Diseases Models and Innovative Therapies) <http://www.idmitcenter.fr> constituent une unité de recherche scientifique et technologique commune entre le CEA, l'Université Paris-Saclay, l'Inserm, l'Institut Pasteur, l'ANRS et la société OncoDesign, spécialisée dans le domaine des maladies infectieuses humaines et des maladies auto-immunes. Les principaux objectifs de recherche et de développement technologique du Département IDMIT/UMR1184 (Directeur: Dr Roger Le Grand), sont de contribuer à mieux comprendre la complexité des relations hôte/pathogène, à exploiter les connaissances acquises pour favoriser le développement de nouveaux médicaments, de stratégies thérapeutiques innovantes et aussi de contribuer au développement des nouvelles générations de vaccins, à développer des modèles précliniques primates non humains de haute valeur prédictive de ce qu'il adviendra chez l'homme en matière d'infection, d'inflammation ou de dysfonctionnements du système immunitaire.

The objective of the PhD project is to better understand the interactions between the HIV/SIV virus and the effector immune cells at the whole body scale and at the cellular scale in nonhuman primates (NHP) using in vivo imaging. This will permit a better understanding of how we can better hit the target and improve the control of viral reservoir and residual replication by using therapeutic strategies against HIV with safer and more potent antiretroviral drug combinations.

The aims of the PhD thesis project will be 1) to localize (in space and in time) the most active replication sites of SIV in NHP using immunoPET-CT technology for whole body distribution; 2) to localize the most active concentration of effector immune cells in response to infection; 3) to focus on the lymph nodes by in vivo two photon microscopy to visualize the dynamics of the host/SIV interactions; 4) to complement the analysis by ex vivo analysis, such as multiparametric confocal microscopy and flow cytometry.

We expect to establish the whole body distribution of the SIV virus and the effector immune cells in infected NHP at different stages of the disease and more specifically, we expect to establish the dynamics of host/SIV interactions in the lymph nodes. This project is part of a program of the Rhiviera consortium, coordinated by the ANRS (Paris, France), aiming to increase our knowledge on drugs' diffusion into different tissues and to assess their concentration levels in cells targeted by HIV, including monocyte/macrophages, dendritic cells and subsets of CD4+ T cells. The present project will then be a complement this program by focusing on the interactions between SIV virus and the effector immune cells, more specifically in the lymph node.

Main tasks

SIV infection follow up in macaques, monitoring of immune response to infection, in vivo imaging in NHP: PET-CT and two-photon microscopy in infected NHP, immunohistology

Laboratory:

*Center for Immunology of Viral, Auto-immune, Hematological and Bacterial diseases
UMR1184 (Université Paris-Saclay, Inserm, CEA), IDMIT Department <http://www.idmitcenter.fr>
Direction de la Recherche Fondamentale
Institut de Biologie François JACOB
Commissariat à l'énergie atomique et aux énergies alternatives
92265 Fontenay-aux-Roses*

**Please send your CV and a letter of motivation to: Catherine Chapon
catherine.chapon@cea.fr**

Context

Increasing evidences are making remission an achievable goal for new anti-HIV therapies [1-4]. Safer and more potent antiretroviral drug combinations can significantly improve control of viremia and open the way for new strategies aiming at minimizing the viral reservoir. Patients in remission have certainly benefited from therapies that limited the size of viral reservoir, down to the level preventing post-treatment rebound of viremia. Early control of this reservoir, limitation of pharmacological sanctuaries and residual replication that can be improved by direct antiviral effects are among the key scientific challenges to make remission a reality for a majority of patients. Finding how we can better hit the target requires whole body understanding of viral/host/drugs interactions. Achieving sufficient drug levels in tissue hosting reservoir cells or with low level of virus production is certainly an important stake for therapeutic improvement. This should be combined with a better understanding of repertoire of infected cells and their distribution in the host. HIV cell targets may have diverse mechanism for drug penetration, metabolism and efflux that change with tissue micro-environment. Also HIV infection affects several physiological functions like cells homing and activation as well as chronic systemic inflammation. The impact of these dysfunctions, especially, chronic inflammation on drugs efficacy remains poorly understood. Tissue investigation using histopathological assays, like immunohistochemistry, has provided critical information regarding the impact of HIV/SIV on the organization of the human immune system at a tissue level [5],[6-11]. These methodologies cannot address the tissue distribution/localization of lymphoid populations in vivo, as well as the anatomical context in which their highly dynamic interactions occur. Therefore, animal models are critical to this research area. Nonhuman primates (NHP) are particularly relevant here. These species share very similar physiological functions with humans and can be infected with pathogenic SIV or SHIV that recapitulate many of the characteristics of HIV infection and AIDS in humans [12]. New approaches to directly visualize viral replication in the host are rapidly emerging, like PET-CT [13-15]. The main objective of the project will be to localize in space and time the most active replication sites of SIV using PET-CT technology and two-photon microscopy in infected NHP at different stages of the disease. The ultimate goal will be to obtain a map of the interactions between the virus distribution and the host at whole body and cellular resolution in order to better understand how to better hit the target by improving drug administration.

Objectives

The objectives of the PhD project will be :

- 1) To localize (in space and in time) the most active replication sites of SIV in NHP using immunoPET-CT technology for whole body distribution
- 2) To localize the most active concentration of effector immune cells in response to infection
- 3) To focus on the LNs by in vivo two photon microscopy to visualize the dynamics of the host/SIV interactions
- 4) To complement the analysis by ex vivo analysis, such as multiparametric confocal microscopy and flow cytometry

Methods

- 1) *To localize (in space and in time) the most active replication sites of SIV in NHP using immunoPET-CT technology for whole body distribution.*

In vivo imaging approaches will be developed to study whole body distribution of SIVmac251 antigens in NHP by immunoPET approach. Total-body SIV antigens distribution in NHP will be

characterized using antibody-targeted positron emission tomography (immunoPET technology; Santangelo et al. 2015). In our study, we will use the zirconium 89 for antibody coupling as it shows a longer decay half-life than the copper 64 (3.3 days vs 12.7h) more compatible with the relative slow pharmacokinetics of intact antibodies. This will enable the labeled antibody to achieve high signal-to-background ratios before decay. We will use this approach to characterize the viral replication on cynomolgus macaques infected with SIVmac251 virus at different stages of the disease. Ex vivo analysis on tissues will be performed using autoradiography, immunohistology and qRT-PCR to correlate the in vivo results obtained by imaging.

2) *To localize the most active concentration of effector immune cells in response to infection*
Alternatively with immunoPET-CT imaging to characterize virus distribution, we will be able to characterize the distribution of effector immune cells (T cells, macrophages, antigen presenting cells, ...) in SIV infected NHP at different stages of the disease by immunoPET. This will allow us to compare the whole body distribution of the virus with the whole body distribution of selected subsets of effector immune cells at a specific stage of the disease. Specific antibodies targeting effector immune cells will be labelled with ⁸⁹Zr and will be injected intravenously. PET-CT imaging will be performed in healthy control NHP and SIV infected NHP in order to assess the evolution of some specific effector immune cells, which are also the major targets of HIV/SIV infection, such as CD4 T cells and antigen presenting cells (HLA-DR) with macrophages (CD163, CD68) and dendritic cells (CD11c). The imaging results will be validated against histopathological biomarkers.

3) *To focus on the LNs by in vivo two photon microscopy to visualize the dynamics of the host/SIV interactions upon repeated imaging sessions*

In order to focus on the distribution of the virus in the lymph nodes by two-photon microscopy, fluorescent labeled SIV Gp120-specific antibody will be injected (sc or id) in SIV infected animals. Using this technology, we will be able to characterize virus / host interactions not only by labeling and tracking the virus but also by labeling the immune cells after injection of a combination of fluorescent specific antibodies. This will allow the characterization of the local interplay between the virus and host cells, with respect to understanding viral dynamics and persistence, local immune responses and changes to the surrounding milieu and function of immune cells.

4) *To complement the analysis by ex vivo analysis such as multiparametric confocal microscopy and flow cytometry*

Ex vivo analysis on tissues will be performed using gamma counting, autoradiography to correlate the in vivo results obtained by imaging. qRT-PCR will also be performed to evaluate the concentration of the virus in relevant tissues. Furthermore, multiparametric confocal microscopy in relevant tissues will be performed to characterize the different cell subsets interacting with the virus, and the modification of the immune cell distribution in the LN depending on the stage of the disease.

Expected results

We expect here to develop a method allowing the study of whole body SIVmac251 antigens distribution in combination with the characterization of the distribution of effector immune cells in NHP with little invasive approaches, which do not require tissue biopsies and euthanasia. A particularly innovative aspect of the project will be to associate two in vivo imaging modalities at the whole body and cellular scales to characterize the interactions between the virus and the effector immune cells and have an overall picture of the dynamics of the virus/host interactions.