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Abstract

The laboratory is dedicated to research on HIV and HCV, with an emphasis on host-virus relationship and studies on virus entry. The main projects concern : Role of lipids in spatial distribution of viral receptors and co-receptors on the plasma membrane. HIV-envelope-dependent cell fusion assays in resting lymphocytes, and testing of entry inhibitors. Expression of HCV envelope proteins. Creation of original, human cDNA microarrays to characterise lymphocyte transcriptional responses to HIV. Lectin receptors , and their role in viral transfer/dissemination. Real-time imaging of early events following cell infection.

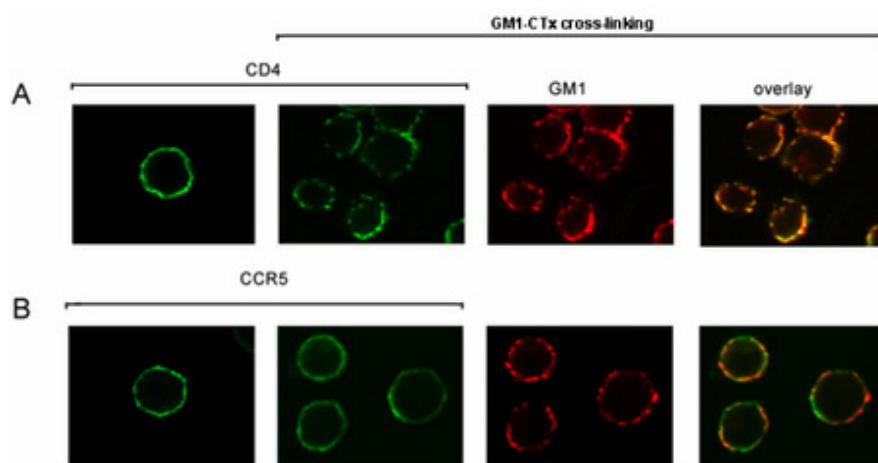
Annual Report

Post-transduction regulation of the chemokine SDF-1 and its receptor CXCR4. Contribution to the study of the regulation of bone marrow homing of CD34+ cell precursors. F. Arenzana-Seisdedos, A. Valenzuela, T. Planchenault, I. Staropoli.

The pair SDF-1/CXCR4 is unique among chemokines/chemoreceptors for its pleiotropic activity that concerns many aspects of embryo development and organogenesis, the regulation of leukocyte traffic or the inhibition of CXCR4-tropic HIV viruses. SDF-1/CXCR4 is critically involved in the homing and retention of CD34+ precursors in the bone marrow where SDF-1 is abundantly expressed by stromal cells. G-CSF-induced egress from bone marrow to periphery is currently used to isolate and purify large amounts of these cells to facilitate engraftment in patients requiring reconstitution of bone marrow hematopoietic functions. We raised the hypothesis that G-CSF may set in motion, directly or indirectly, a proteolytic mechanism which could eventually affect the stability and functions either of SDF-1 or CXCR4. In collaboration with D. Pidard and M. Chignard (IP), we showed that leukocyte elastase selectively cleaves the amino-terminal domains of both SDF-1 and CXCR4, thus abolishing their capacities to activate the receptor and bind the ligand, respectively. With our collaboration, those findings have been validated by other groups (Petit *et al*, *Nature Immunology* , 2002; Levesque *et al.*, *J. Clin. Invest* , 2003) and our hypothesis was confirmed both in experimental animals *in vivo* , and *ex vivo* in samples collected from CD34+ donors injected with G-CSF. In conclusion, the pharmacological effect of G-CSF in the mobilisation of CD34+ cells is largely accounted by a finely tuned mechanism , likely to depend on the release of proteolytic enzymes, particularly elastase, released by granulocytic cells and opposing cell anchoring/cell signaling provided by the pair SDF-1/CXCR4.

Spatial distribution of HIV receptors inside and outside lipid rafts, and its relevance to HIV entry F. Bachelerie, Y. Percherancier, T. Planchenault, I. Staropoli, B. Laganne.

HIV fusion with cell membranes depends on the successive interaction of the viral envelope with CD4 and a chemokine receptor (CCR5 or CXCR4). Investigating the molecular mechanisms that underlies the lateral distribution on the plasma membrane of the HIV-1 primary receptor CD4 and co-receptors CCR5 and CXCR4 in relation to their function, association with protein networks and interactions in plasma membrane microdomains (or "rafts") are critical elements for the comprehension of early steps of HIV entry into target cells. A main question is to know whether CD4 and the co-receptors are in intimate contact in discrete domains of the cell membrane. F. Bachelerie and her colleagues dissected the contribution to virus-cell attachment and fusion of receptor localization inside or outside lipid microdomains, where CD4 partly distributes. The realization of this program relied on a combination of biochemical / cellular approaches and real-time biophysical and microscopy methods carried out in live cells. Having shown previously that CCR5 does not co-localize into rafts in spite of its modification by palmitoylation (J.B.C., 2001), the team identified the determinants that address a fraction of CD4 to specialized membrane microdomains Using expression of CD4 mutants, evidence was provided that HIV-1 entry does not depend upon CD4 distribution into rafts ,or its association with the Src kinase p56^{Lck} . It was also shown that membrane cholesterol contents modulate HIV entry independently of the ability of cholesterol to promote raft formation (J.B.C., 2002).



HIV fusion in resting CD4 T lymphocytes: a novel mechanism for creating viral reservoirs ? R. Altmeyer, P-Y Lozach, I. Staropoli, C. Chanel, de Lacroix de Lavalette

A main therapeutic problem in HIV-infected people is that antivirals efficiently suppress virus replication, but cannot eradicate the virus, the replication of which resumes as soon as therapy is interrupted. Thus, some kind of "reservoir(s)" must be postulated, where HIV persists in an infectious form without replicating. Ralf Altmeyer and his colleagues have used an original fusion assay permitting to investigate whether envelope-dependent HIV entry takes place in resting lymphocytes , that is in cells non-permissive to HIV genome transcription. This "FLASH" assay does not rely on transcription of an HIV-promoter-dependent reporter gene, but rather measures by cytofluorometry

analysis the fusion process itself. It was observed that peripheral CD4 cells in a state of complete rest express minute, yet functional amounts of membrane CCR5. This co-receptor is used by HIV to fuse with the cell membrane. Such results are consistent with the hypothesis that HIV enters into non-activated, non-permissive CD4 lymphocyte, where its genetic material is not transcribed until a later

activation process induces permissiveness to virus replication . This strategy may allow HIV to escape both immune recognition and eradication by available antiviral drugs.

This fusion assay is now performed in microplates, permitting the high-throughput screening of molecules blocking HIV entry.

CCR5 receptor signaling through G-proteins is not required for efficient HIV infection in lymphocytes and macrophages F. Arenzana-Seisdedos, A. Amara, A. Vidy

It has long been assumed that HIV uses the co-receptor CCR5 not only as an anchoring molecule to induce fusion, but also as a transmembrane molecule capable of signaling through heterotrimeric G-protein, which would modify the cell and render them permissive to HIV-genome transcription and replication. We have taken advantage of the availability of leukocytes from people genetically deficient in CCR5 expression (delta-32 deletion) to induce expression of mutant CCR5 gene unable to couple to G-proteins in otherwise normal leukocytes. Expression of such CCR5 mutants did not induce calcium mobilisation upon agonist-induced triggering, yet provided full co-receptor function. Replication occurred as efficiently as in wild-type CCR5-transfected cells. This indicated that in a primary, CCR5-reconstituted CD4 cell environment, G-protein signalling is dispensable for R5 HIV isolates to actively infect normal T lymphocytes or macrophages.

Investigations on the pathogenicity of HIV/SIV infection L. Chakrabarti, E. Cabannes, L. Laurent

Studies of natural SIV infection indicate that virus replication is necessary, but not sufficient to induce immunodeficiency in primates. A central question in understanding the pathogenic mechanisms of AIDS is thus to characterize the molecular mechanisms that render CD4 lymphocytes prone to programmed cell death, and lead to their progressive depletion . We are testing the hypothesis that HIV envelope glycoprotein transduces signals through its receptor CD4 and coreceptors CCR5 or CXCR4, a phenomenon which may play a role in the abnormal activation and poor survival characteristic of CD4 lymphocytes in infected patients. Lisa Chakrabarti analyses the role of Env-induced signals in abnormal T cell activation by dynamic imaging and biochemical approaches in primary cell culture systems. The very early events induced by binding of HIV and SHIV Env to the CD4/coreceptor complex are investigated by using the relocalisation of GFP fused to key signaling proteins (AKT, CD3-zeta) as a readout. To explore the possibility that Env perturbs the process by which antigen presenting cells (APC) activate naive T cells, we will analyze the effect of purified Env and of virion preparations on the formation of immunological synapses at the junction between APC and T cells. This work is performed in close collaboration with the imaging core facility of the institute (CID, S. Shorte)

Another approach to the question (Eric Cabannes) is to use human cDNA spotted on glass microchips to characterise the pattern of genes induced or repressed in CD4 lymphocytes upon in vitro interaction with either HIV or the chemokine SDF-1, the natural ligand of the CXCR4 co-receptor. Such long-term investment on genomics is made in collaboration with two microarrays core facilities, that of the french Genopole (Evry) and that of the Institut Pasteur (J-Y Coppée).

Both approaches on pathogenicity are lead in parallel progress , and have inspired the project of a General Transverse Programme (GPH) of Institut Pasteur on HIV/SIV pathogenicity and the role of virus-induced signals in de-regulating T cell functions.

Role of lectin receptors in viral infection and dissemination F. Arenzana-Seisdedos, A. Amara

In collaboration with colleagues in Bordeaux, the laboratory has shown that interaction of the human cytomegalovirus envelope with the lectin receptor DC-SIGN is critical for infection of dendritic cells. Moreover, the CMV Env/DC-SIGN interaction was essential for target cell trans-infection.

A similar approach is now being followed to study the pathogenesis of HCV and dengue infections. We have also participated in the demonstration that DC-SIGN is the major Mycobacterium tuberculosis receptor on human dendritic cells.

Coordination : The various approaches described above are coordinated by Fernando Arenzana-Seisdedos and Jean-Louis Virelizier

Legend : **In contrast to CD4, patching of GM1 with Cholera toxin (CT) does not induce clustering of CCR5.** Aggregation of GM1 clusters was initiated by incubation of the CTx-PE-labeled A3.01R5 cells with CTx antibody (GM1 panels). CD4 (A) and CCR5 (B) staining in absence (left panels) or upon GM1 clustering (right panels) are shown. The merge of the GM1 and CD4 (A) or CCR5 (B) signals are also presented.

Keywords: HIV,AIDS, HCV,hepatitis, receptors,lectin, fusion, pathogenesis

Publications

> [Publications of the unit on Pasteur's references database](#)

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