ImmunoTools *special* Award 2021

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The role of CXCR4 dynamics in the entrance of HIV-1

Human Immunodeficiency Virus (HIV-1) infection is characterized by a persistent $CD4^+$ T lymphocyte destruction which ends in a chronic and progressive immunodeficiency of the affected individual. It represents one of the main challenges for public health. In 2019, the number of people infected by the virus increased up to 38 million worldwide. The entrance of the virus in their target cells depends on the interaction of the viral glycoprotein, gp120 with CD4, followed by interaction with a chemokine coreceptor (CXCR4 or CCR5). R5 viral strains use CCR5 as coreceptor, replicate in peripheral blood mononuclear cells (PBMCs) and macrophages and are predominant at early stages of the infection. X4 strains use CXCR4 as coreceptor, replicate in PBMCs and T lymphocytes and usually emerge during infection progression. Finally, there are X4/R5 or dual tropic strains, in which the virus can use both coreceptors, CXCR4 or CCR5, affecting all cell types mentioned.

Chemokine receptors belong to seven transmembrane G protein-coupled receptor (GPCR) family. They are formed by a single polypeptide chain that crosses seven times the plasma membrane. These receptors have an extracellular domain implicated in ligand binding and an intracellular one, whose function is binding signaling proteins. After ligand binding CKRs, several signaling pathways are activated producing a cellular response based on the expression of different genes, mobilization of intracellular calcium reserves, cellular adhesion, chemotaxis events, cell polarization, among others.

CKRs are often detected as individual receptors as well as receptor complexes in the presence of their ligands. Improvements in biophysical techniques and advances in microscopy have allowed the characterization of CKRs distribution and dynamics in the membrane of live cells, showing that CKRs could be in monomers, dimers, oligomers or clusters. The aggregation level of CKRs can modulate their response (ligand affinity, signal transduction, internalization process, cell activation and migration).

The main objective of our project is to study the influence of the aggregation level and the dynamics of CXCR4 in the infection by HIV-1, and how the modulation of these characteristics could modify the entrance of the virus in target cells.

We are using Jurkat cells transfected with CD4 and CXCR4 bound or not to fluorescence proteins, treated with a recombinant X4 tropic gp120 protein, lentiviral particles that express the viral glycoprotein or the natural ligand of CXCR4, CXCL12. To elucidate which is the effect of these ligands in CXCR4 dynamics, we use resonance energy transfer (fluorescence FRET or bioluminescence BRET) and confocal microscopy techniques (total internal reflection fluorescence microscopy). To evaluate how changes in CXCR4 dynamics affect the cell function, we are using Jurkat cells and primary $CD4^+$ T lymphocytes in multiple assays, including intracellular calcium flux, cell polarization, chemotaxis and adhesion. The main activation signaling pathways are analyzed by western blot.

To evaluate if the dynamics of CXCR4 affect HIV-1 entrance we will perform cell fusion experiments using Jurkat cells expressing gp120 viral protein and Jurkat cells which co-express CD4 with wild type CXCR4 or a mutant CXCR4 unable to form larger clusters upon ligand activation.

Taking into account this overview, for us it is very interesting to participate in your grants. We are mainly interested in anti-human APC-CD3, FITC-CD4 and PE-CD8 antibodies labelled with different fluorochromes (PE, FITC) to characterize our cell lines and primary cells, recombinant human IL-2 for activation of primary cells and recombinant human CXCL12a and b as CXCR4 ligand.

ImmunoTools *special* AWARD for **Adriana Quijada** includes 10 reagents

FITC - conjugated anti-human CD3, CD4, CD8

PE - conjugated anti-human CD4, CD8

APC - conjugated anti-human CD3, CD8

recombinant human rh IL-2, CXCL12a and b as CXCR4 ligand

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