The Sphingosine-1-Phosphate Receptor 1 Dimerizes with CCR5 and Enhances HIV Infection

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Background: CCR5 is a G protein-coupled receptor (GPCR). GPCR dimerization may result in a change in cell surface density, in ligand specificity and affinity and in the nature and intensity of the signaling pathway. Therefore, we looked for GPCR able to dimerize with CCR5 at the CD4 T cell surface.

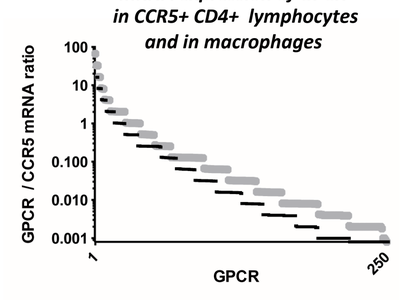
Methodology: We identified by multi quantitaiive RT-PCR the GPCR coexpressed with CCR5 in circulating CD4 T cells. GPCR dimerization was evidenced by time-resolved FRET. Using HIV gene transfer vectors, we established two cell lines expressing or not the sphingosine-1-phosphate receptor 1 (S1P1). HIV infectibility by replicative and non-replicative HIV-1 strains was measured in both cell lines. The effect of S1P1 expression on LTR activation was studied in cells stably transfected with a reporter gene driven by HIV LTR and transduced with the S1P1 gene. S1P1 expression at the surface of primary cellswas measured by flow cytometry. The effect of the S1P1 antagonist FTY720 was tested on SCID mice reconstituted with human peripheral blood mononuclear cells (PBMC) and infected with the HIV-1 R5 strain JR-CSF.

Results: We identified 250 GPCR coexpressed with CCR5 in circulating CD4 T cells. Among the GPCR the most expressed, we found that S1P1 dimerized with CCR5 at the cell surface. S1P1 coexpression resulted in a decrease in HIV-1 R5 virion entry, but not in cell to cell propagation. By contrast, S1P1 coexpression boosted LTR activation. Altogether these effects resulted in the increase in replicative HIV-1 R5 infection. We detected S1P1 at the surface of monocyte-derived dendritic cells, and FTY720 inhibited R5 infection in these cells. Moreover, this S1P1 antagonist inhibited HIV-1 R5 infection in a mouse model.

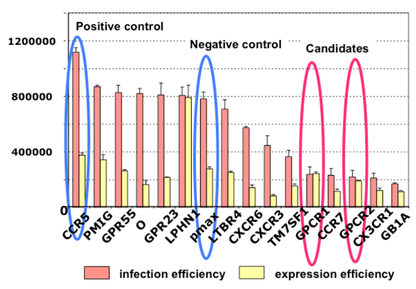
Conclusions: S1P1 dimerizes with CCR5 and its expression increases HIV-1 R5 replication. Consequently, an S1P1 antagonist inhibited R5 infection in vitro and in vivo. S1P1 might also impair the chemokine receptor function of CCR5, and vice versa.

Identification of S1P1 by screening for GPCRs that interfere with HIV-1 infection

mRNA expression of GPCR in CCR5+ CD4+ lymphocytes and in macrophages



Testing the effect of GPCR expression on HIV-1 infection in CD4+ CCR5+ HEK293 cells



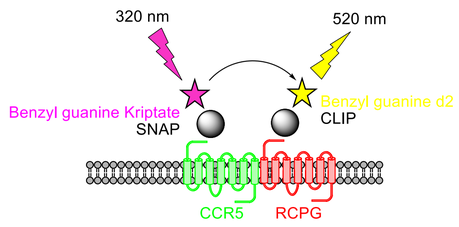
S1P receptor	S1PR / CCR5 in CCR5+ CD4+ lymphocytes	S1PR/ CCR5 in macrophages
S1P1/EDG1	33	0.1
S1P2 /EDG5	2	2
S1P3/EDG3	0,04	0.01
S1P4/EDG6	0,5	0.1
S1P5/EDG8	2	0.03

S1P1 reduces infection of HEK293 cells by a one-round virus

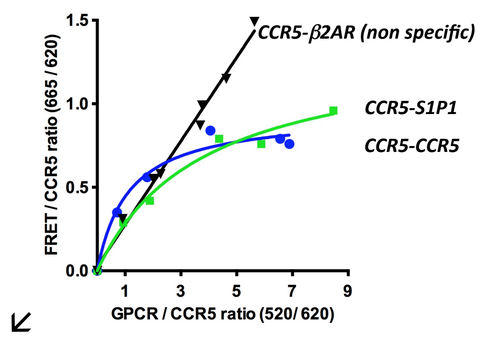
→ S1P1 mRNA is highly expressed in CCR5+ CD4+ lymphocytes

S1P1-CCR5 interaction at the cell membrane observed by time-relapse FRET

Tr-FRET partners



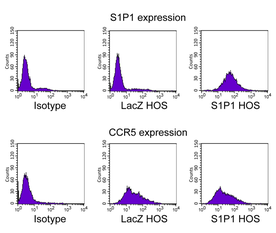
Tr-FRET saturation assay



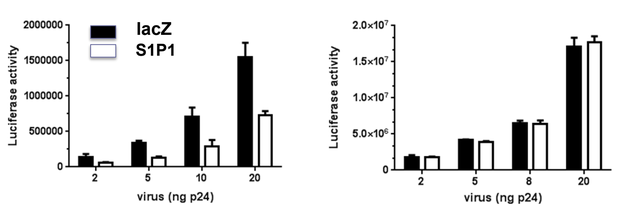
S1P1 and CCR5 form heteromers at the cell membrane

Effect of S1P1 on the first cycle of HIV-1 replication in HOS cells

S1P1 membrane receptor expression after transduction in CCR5+ or lacZ+ HOS cells

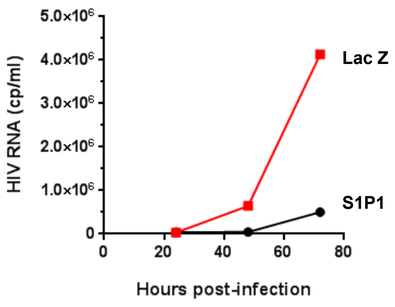


Infection levels of CCR5+ lacZ+ HOS versus CCR5+ S1P1+ HOS cells by a one-round virus



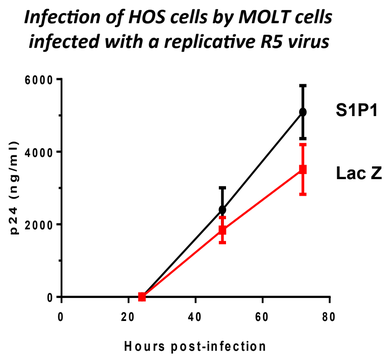
S1P1 decreases viral entry through CCR5

Infection by a replicative R5 virus



→ S1P1 inhibits the first cycle of HIV replication by free viral particles

Infection of HOS cells by MOLT cells infected with a replicative R5 virus

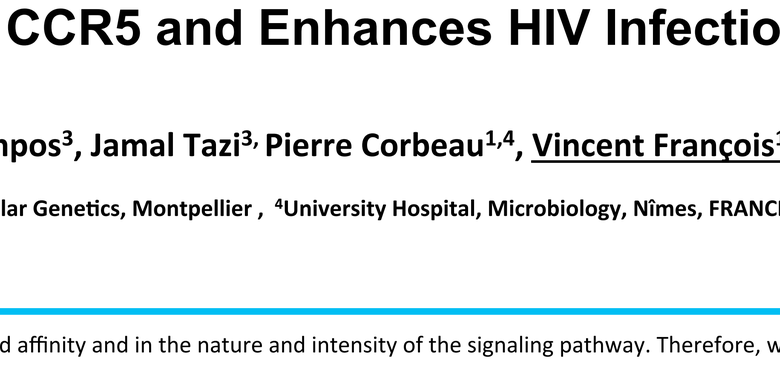


← S1P1 enhances a single cycle of viral production when by cell-to-cell contact is the mode of infection

S1P1 inhibits the first cycle of HIV replication at the level of entry when infection occurs by free viral particles, not by cell-to-cell contact.

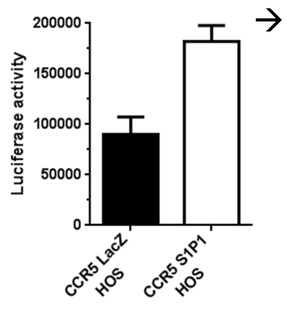
Effects of S1P1 over several virus cycles

Infection levels of CCR5+ lacZ HOS versus CCR5+ S1P1 HOS cells by a replicative virus.



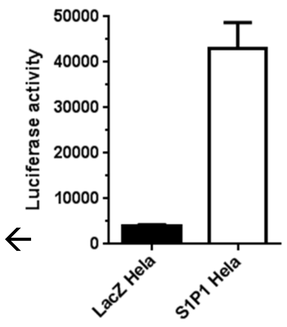
S1P1 increases HIV production in the course of a replicative virus

S1P1 expression is delayed to occur only after entry of the R5 one-round virus



→ S1P1 increases HIV post-entry production

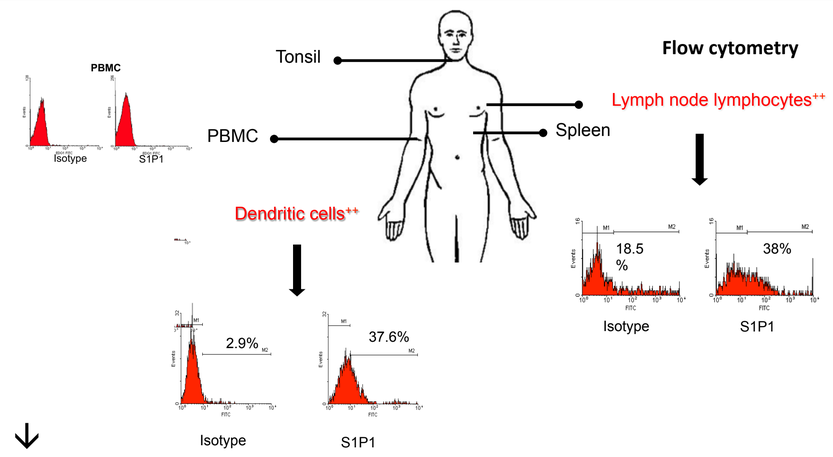
LTR-luciferase activity in Hela cells



← S1P1 activates HIV-LTR expression in HeLa cells

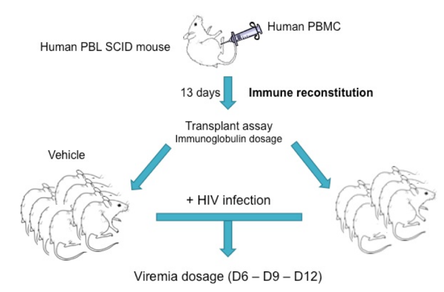
S1P1 increases HIV production by stimulation of HIV transcription

S1P1 expression at the surface of immune cells

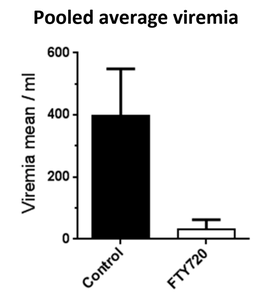


S1P1 is not detected at the surface of PBMC, macrophages and spleen lymphocytes, … but is detected on lymph node lymphocytes and differentiated dendritic cells.

Effect of the S1P1 functional antagonist FTY720 on infection *in vitro* et *in vivo*

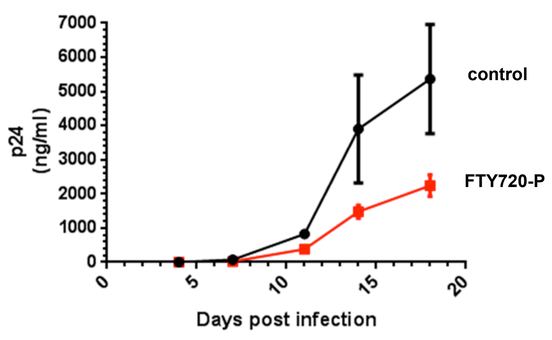


→



FTY720 reduces plasma viral load of infected humanized SCID mice.

Infection of (Vpx treated) dendritic cells (DC) by a R5 replicative virus after exposure to FTY720p



→ FTY720 reduces replicative R5-HIV infection of immune cells (DC) expressing S1P1 at the membrane