Differences in HIV Receptor Activity of CXCR4 Isoforms: Consequences for X4 Strains Emergence

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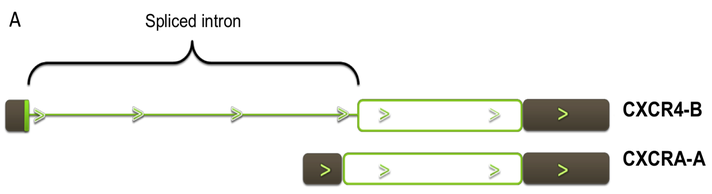
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Background: Two CXCR4 isoforms have been described in humans, CXCR4-A and CXCR4-B, corresponding to an unspliced and a spliced mRNA, respectively. In this study we analyzed the expression of both isoforms, and their functions as HIV coreceptors and chemokine receptors.

Methodology: To this aim, using HIV gene transfer vectors, we established two cell lines expressing each isoform. HIV infectibility by replicative and non-replicative X4 strains was measured in both cell lines, and the amount of early reverse transcripts was quantified as a marker of viral entry. The capacity of both cell lines to support R5 to X4 switch was studied in vitro. Chemotaxis assays were performed. Expression of the two forms of CXCR4 mRNA was evaluated by specific RT-PCR. R5/X4 genotype was defined in HIV-infected persons by determining the False-Positive Rate (FPR) score calculated by the Geno2Pheno algorithm using the sequence of the V3 region of the envelope.

Results: We showed that CXCR4-B, but not CXCR4-A, mediates an efficient HIV-1 X4 entry and productive infection. Yet, the chemotactic activity of CXCL12 on both isoforms was similar. In vitro infection with an R5 strain increased CXCR4-B : CXCR4-A mRNA ratio in peripheral blood mononuclear cells (PBMC), and this ratio correlated with HIV RNA plasma level in R5-infected individuals. In addition, the presence of the CXCR4-B isoform favored R5 to X4 switch more efficiently than CXCR4-A in vitro. Moreover, the capability of circulating HIV-1 strains to use CXCR4, determined after the FPR, was linked to the predominance of CXCR4-B over CXCR4-A expression in PBMC. Finally, we achieved a specific siRNA-mediated knockdown of CXCR4-B.
Conclusions: CXCR4 isoforms present with similar chemokine receptor but different HIV coreceptor capabilities. Our data support the hypothesis that HIV-R5 infection favors CXCR4-B expression over that of CXCR4-A, a phenomenon that could facilitate R5 to X4 switch. We also established a proof of concept for a possible gene therapeutic approach aimed at specifically blocking the HIV coreceptor activity of CXCR4-B.

The two CXCR4 isoforms from alternative splicing (*Gupta et al., J. Immunol, 1999)*

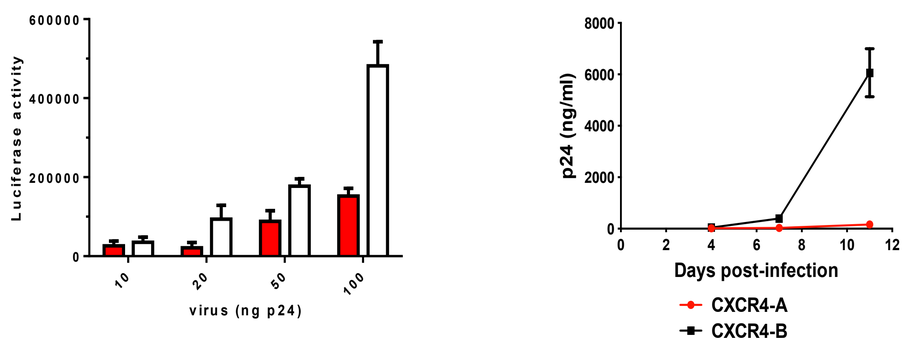


→ CXCR4-A and CXCR4-B differ by a few residues in their extracellular N-terminal domain

Compared efficiency of CXCR4-A and -B as HIV-1 coreceptor in HOS cells

Infection with

a one-round X4 virus **or a replicative X4 virus**

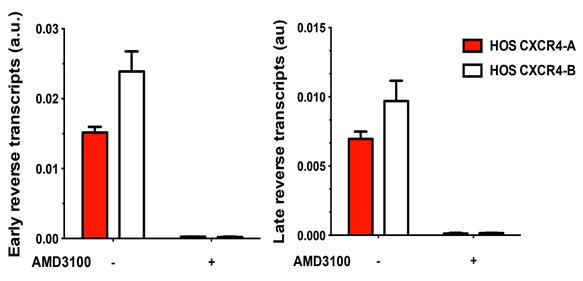


→ Higher infectability of CXCR4-B compared to CXCR4-A HOS cells

No difference between A and B after infection by an R5 or VSVG pseudotyped virus (not shown)

Compared efficiency of reverse transcription after infection of CXCR4-A or –B HOS cells

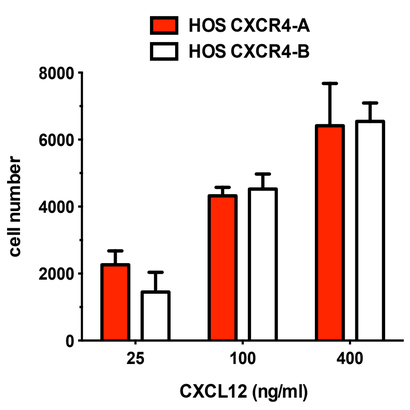
Early transcripts **Late transcripts**



→ More early transcripts are produced in the CXCR4-B cells.
→ The difference between B and A cells is the same for late transcripts.

CXCR4-B allows a more efficient virus “entry”

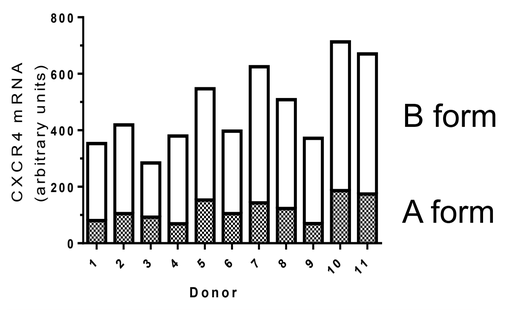
Chemotaxis towards CXCL12 of the two isoforms



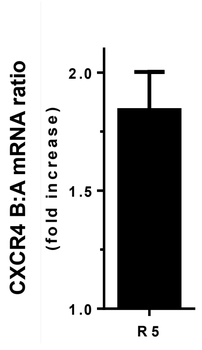
→ No difference in chemotaxis between the CXCR4 isoforms towards their ligand CXCL12

CXCR4-A may be sufficient to ensure the physiological functions

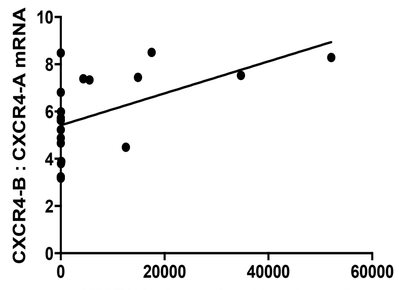
Effect of HIV infection on the B/A ratio in PBMC in vitro and in infected patients



↓ The B form is more abundant in PBMC of healthy donors



→ CXCR4 B/A ratio is increased after infection of healthy donors PBMC with an R5 virus.

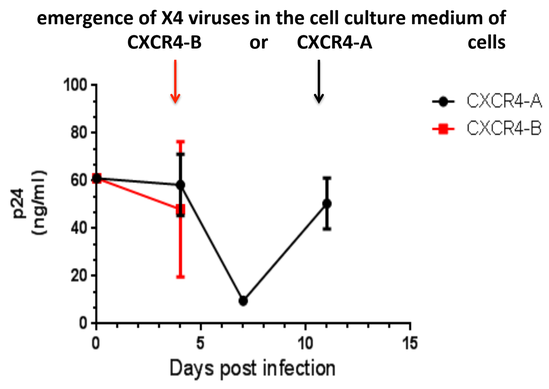


→ Correlation between the B:A ratio and viremia in HIV-1 R5 infected patients

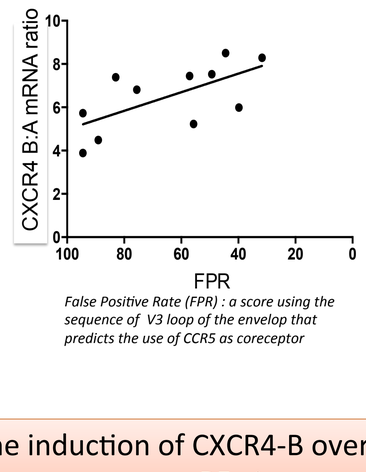
→ R5 infection induces a higher CXCR4-B expression both *in vitro* and *in vivo*

CXCR4-B expression and R5 to X4 switch

CXCR4-B induces the emergence of X4 virions more rapidly than CXCR4-A in R5 HIV-1 infected HOS cells ←



emergence of X4 virions in R5 infected persons



False Positive Rate (FPR) : a score using the sequence of V3 loop of the envelop that predicts the use of CCR5 as coreceptor

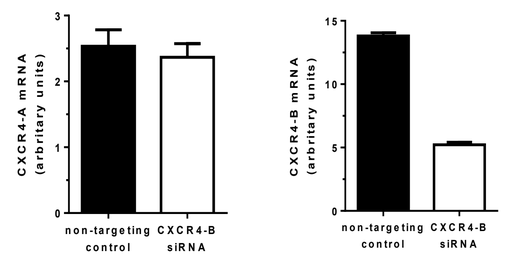
→ A higher risk of R5 to X4 switch (lower FPR) is correlated to a higher CXCR4-B/A ratio in PBMC

The induction of CXCR4-B over CXCR4-A resulting from the infection by an R5 virus may favor the R5 to X4 switch.

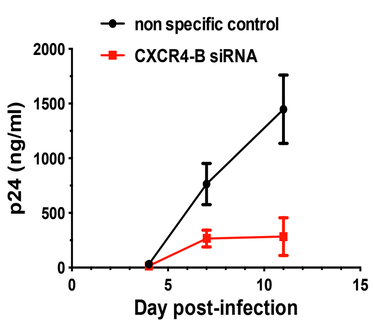
Specific knock-down of CXCR4-B and effect on infectability

Downregulation of CXCR4-B
CXCR4-A is unaffected. ←

Transfection of HeLa P4 cells with CXCR4-B siRNA



Infection of HeLa P4 cells after transfection with the CXCR4-B siRNA



→ Drastic decrease in infectability by a X4 virus

New potential anti-HIV therapy