Phenotype variation in human immunodeficiency virus type 1 transmission and disease progression

Mariangela Cavarelli* and Gabriella Scarlatti

Viral Evolution and Transmission Unit, Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy

Abstract. Human immunodeficiency virus type I (HIV-1) infects target cells through interaction with the CD4 molecule and chemokine receptors, mainly CCR5 and CXCR4. Viral isolates can be phenotypically classified based on the co-receptor they utilize to infect target cells. Thus, R5 and X4 virus use respectively CCR5 and CXCR4, whereas R5X4 virus can use either CCR5 or CXCR4. This review describes the central role played by co-receptor expression and usage for HIV-1 cell tropism, transmission and pathogenesis. We discuss various hypotheses proposed to explain the preferential transmission of R5 viruses and the mechanisms driving the change of HIV-1 co-receptor usage in the course of infection. Recent insights in the intrinsic variability of R5 viruses and their role in influencing disease progression in both adults and children are also discussed.

Keywords: HIV-1, co-receptor usage, tropism, transmission, disease progression

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) entry into the host cell is dependent upon binding of the virus envelope protein (*env*) to the cellular receptor CD4 and a co-receptor, generally the chemokine receptors CCR5 or CXCR4. The identification of chemokine receptors as the co-receptors of HIV-1 improved our understanding of the pathogenesis of HIV-1 infection with respect to the mechanism of viral entry, viral tropism and differences in disease course among infected individuals. CCR5 is the predominant co-receptor exploited for transmission and replication *in vivo*, and R5 viruses are detected at all stages of infection. CXCR4-using virus variants evolve in about one-half of subtype B infected individuals, and their emergence is associated with an accelerated course of the disease. Here we will

discuss the chemokine receptor used by HIV-1 to infect target cells, their relevance to define viral tropism, their involvement in viral transmission, viral phenotypic evolution and disease progression both in adult and paediatric HIV-1 infected patients.

2. Identification of CCR5 and CXCR4 as HIV-1 co-receptors: The history

Almost immediately after the discovery of HIV-1 as the causative agent of HIV/AIDS, the CD4 molecule was identified as the main receptor used by the virus to infect target cells. Early evidence derived from the observation that infection was inhibited with monoclonal antibodies to CD4 [1]. This finding was later confirmed demonstrating that resistant human cells transfected with a recombinant copy of the CD4 gene became susceptible to HIV-1 infection [2,3]. Soon thereafter evidences started to accumulate indicating that CD4 alone was not sufficient to explain HIV-1 tropism for different target cells *in vitro*. Two main observations supported

*Corresponding author: Mariangela Cavarelli, PhD, Viral Evolution and Transmission, San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milano, Italy. Tel.: +39 022643 4907; Fax: +39 022643 4905; E-mail: cavarelli.mariangela@hsr.it.

the notion that a co-receptor was required for HIV-1 entry. The first one was that CD4-transduced human cells were permissive for HIV-1 infection and replication, but transfected murine cells were not, despite appropriate processing and delivery of CD4 to the cell surface [4,5]. The second evidence came from experiments with murine/human cell hybrids that supported the conclusion that HIV-1 required a positive cofactor (co-receptor) specific for human cells for productive infection [6].

Additional evidences that a co-receptor for HIV-1 entry was required, derived from the observation that HIV-1 exhibited *in vitro* distinct cellular tropism for CD4+ human target cells, CD4+ T cells and macrophages, which brought to a first attempt of classification of HIV-1 as T cell line tropic (TCL-tropic) and Macrophage-tropic (M-tropic).

In 1995, the discovery that the chemokines RANTES (regulated on activation, normal T cell expressed and secreted), MIP-1 α and MIP-1 β (macrophage inflammatory protein) had a strong inhibitory effect on some viral strains changed radically the knowledge on viral entry [7]. Shortly thereafter Feng et al., using a novel cDNA cloning strategy based on the ability of a cDNA library to render a CD4-expressing murine cell permissive for fusion with cells expressing *env* from TCL-tropic HIV strains, identified a new protein, defined "fusin" [8]. This protein is a member of the superfamily of the seven transmembrane domain G protein-coupled receptors, and so a putative chemokine receptor, but no ligands or functional activity was found. These observations prompted several groups to investigate the possible involvement of other chemokine receptors in the entry of HIV into cells. On one hand it was soon demonstrated that fusin is indeed a chemokine receptor, specific for the CXC chemokine stromal cell derived factor-1 (SDF-1) α and β , and renamed CXCR4 [9, 10]. On the other hand the chemokine receptor for RANTES, MIP-1 α and MIP-1 β was identified and designated CCR5 [11,12]. CXCR4 was shown to be the main receptor for TCL-tropic HIV strain and SDF-1 a selective inhibitor, and within one week five independent groups demonstrated that CCR5 was the main co-receptor for M-tropic strains [13–16].

These findings consequently fused the two formerly separate fields of HIV-1 and chemokine research and unveiled a fascinating new horizon for the understanding of HIV-1 pathogenesis. Presently a wide array of chemokine and chemokine-like receptors act as co-receptors for HIV-1 *in vitro*, including CCR2b, CCR3, STRL33/Bonzo, GPR1 and GPR15/BOB and

CX3CR1, to name but a few, however, current evidences suggests that co-receptors other than CCR5 and CXCR4 have limited use *in vivo* [17–23]. The factors that preclude the use of a wide range of coreceptors *in vivo*, as well as the reasons why the virus fails to evolve variants capable of exploiting alternative co-receptors, are not yet known.

3. HIV-1 cellular tropism explained by co-receptor usage

Before the identification of chemokine receptors as HIV-1 co-receptors, Asjo et al. described two distinct groups of viruses, defined as slow-low and rapid-high, depending on their replication rates in peripheral blood mononuclear cells (PBMCs) [24]. Further differences in tropism of the virus isolates emerged when CD4+ cells other than T cells were used as target. Some isolates grew well in T-cell lines, but showed poor or no infectivity for primary macrophages, and were defined T-cell line tropic (TCL-tropic). Other virus isolates showed the opposite preference, infecting primary macrophage much more efficiently than continuous T-cell lines, and were defined macrophage-tropic (M-tropic) [25]. The distinction was not always that sharp with isolates capable of infecting both cell targets, called dual-tropic. An important contribution came from the Dutch group which introduced a new definition of the two groups of viruses: syncytium-inducing (SI) or non syncytium-inducing (NSI) according to their capacity to induce cytopathic effect of the permissive T-cell line MT-2 [26]. The genetic determinants for the two types of viral phenotypes were localized in the gp120 *env* sequence and predominantly in the V3 loop (for a review see [27]).

The identification of CCR5 and CXCR4 as the main HIV-1 co-receptors explained the difference in cell tropism of the virus isolates as well as their sensitivity to the natural ligands of these receptors, the chemokines RANTES, MIP-1 α and MIP-1 β , or SDF-1, respectively. NSI variants use CCR5 (expressed by primary macrophages and primary T cells), while SI variants use CXCR4 (primarily expressed by continuous T cell lines and primary T cells) [8,13–16,19]. Thus, a new nomenclature has been proposed in 1998 [28]: viruses using CCR5 were designated R5, whereas those using only CXCR4 were nominated X4 or R5X4 when able to use either co-receptor (Fig. 1).

Recently, Karlsson et al. deeply explored the intrinsic variability of the replication capacity and the sensitivity

HIV-1 phenotype

Fig. 1. HIV-1 tropism. R5 viruses replicate efficiently in primary CD4+ T lymphocytes and macrophages, but not in CD4+ T cell lines. X4 and R5X4 viruses replicate in primary CD4+ T lymphocytes, macrophages and CD4+ T cell lines.

to β -chemokines of R5 viruses. They infected a panel of U87.CD4 cells expressing a series of chimeric receptors between CCR5 and CXCR4 (called FC-1, FC-2 and FC-4b) with R5 viruses obtained from HIV-1 infected individuals throughout disease progression [29]. They were able to propose a more subtle classification of CCR5-using viruses according to their ability to use exclusively the *wild type*-CCR5 (named R5^{narrow}), or also different combinations of the CCR5/CXCR4 chimeric receptors (named R5^{broad}). Depending on the number of chimeric receptor used, the R5^{broad} phenotype could be further divided into R5^{broad(1)}, R5^{broad(2)} or R5^{broad(3)}.

4. Co-receptor use and cell tropism are related but distinct Env's characteristics

Although cellular tropism is generally explained by coreceptor usage, there are some exceptions that make the concept of tropism controversial. The co-expression of the CD4 molecule and selected co-receptors have identified monocytes, macrophages, microglia, dendritic cells (DC), Langerhans cells and lymphoid cells such as thymocytes and CD4+ T cells as potential target cells for HIV-1. However, the mere co-expression of the appropriate receptors does not warrant the capacity of a cell to support productive infection.

Primary human macrophages express CCR5 and at moderate levels CXCR4 [30–32], however a complex infectivity pattern has been observed. Indeed, most R5 isolates are able to infect macrophages [33–38]. However, HIV-1 isolates that use CCR5 but yet fail to replicate in macrophages exist [39,40], suggesting a post-entry inhibition of the replication. A better understanding of the viral and cellular factors, required for productive HIV-1 replication and infection, may facilitate the discovery of new and exploitable therapeutic avenues.

The capacity of X4 viruses to infect macrophages is a long lasting controversial issue. The differences observed between different studies could probably be ascribed to different preparation of the macrophages. However, macrophages can be resistant to infection with X4 isolates, despite the presence of CXCR4 on their surface [41,42]. The relatively low level of expression of the CD4 molecule on macrophages together with a higher CD4 dependency of X4 than R5 variants, may lead to a less efficient entry of X4 viruses into macrophages [43,44]. Evidence for this explanation comes from studies, in which over-expression of CD4 rendered macrophages more permissive to infection with primary X4 viruses [45,46]. Additional evidences suggest that primary X4 isolates [47,48], as well as some R5X4 viruses [32,49,50] may infect different types of macrophages.

It has also to be highlighted that additional factors may impact viral tropism, like receptor density, conformation heterogeneity, and post-translation modifications of the chemokines receptors (reviewed in [51]). All of them render the question of how HIV-1 *env* interacts with the co-receptors more complex than the mere definition of the co-receptor usage by any given viral strain. Indeed, it is known, that accessory genes can, at least *in vitro*, interfere with cell tropism. An example are *vpr*, *vpu* and *vif*, accessory genes dispensable for infection and replication in T cell lines, which instead are required to various extend for infection of primary macrophages and PBMCs [52–54].

5. Relevance of co-receptor usage in HIV-1 transmission and establishment of infection

Viral co-receptor usage is known to play a critical role in transmission of HIV-1 infection. *In vivo*, R5 variants are preferentially transmitted, independently of the transmission route, in both adults and children [42,55–61]. X4 viruses are rarely recovered from newly infected individuals [58,62,63], also when such variants are present in the transmitting source. These results suggest that CCR5 usage plays an important role during viral transmission and initial dissemination in the host, and that some restriction to transmission of X4 viruses may exists.

The presence of R5 variants during early stages of HIV-1 infection suggests that macrophages may be one of the principal targets for the establishment of infection after transmission [60,61]. Analogously, DCs and Langerhans cells, abundant at mucosal sites, have also been implicated in transmission [64–68]. A study conducted with rhesus monkeys infected with simian immunodeficiency virus (SIV) *via* vaginal exposure, identified Langerhans cells as the first cellular target [69]. These cells capture the antigen at peripheral tissue level and transport it to lymphoid organs for presentation to T cells. The preferential expression of CCR5 on DCs could explain why the majority of transmission events are mediated by R5 viruses. Although there has been some initial controversy whether HIV-1 can productively infect DCs [70], it has been extensively demonstrated that the specific C type lectin DC-SIGN, expressed on immature DCs, allows capture of the virus and infection through a CCR5-dependent mechanism (*cis* infection). DCs can capture the virus also through DC-SIGN/gp120 interaction and efficiently transfer it

to CD4+ T cells (*trans* infection), without becoming infected [71].

An alternative explanation for the predominance of R5 variants early in infection could be offered by the differential tropism of R5 and X4 variants for different lymphocyte subsets [72]. *In vivo* CCR5 is mainly expressed on activated memory T cells [73–75], while CXCR4 predominates on naïve and resting memory T cells [74]. DCs reaching the gut-associated lymphoid tissue (the main site of early HIV replication in both humans and macaques) induce activation of CD4+ T cells, which acquire a memory phenotype [76–79]. Indeed, *in vivo* and *in vitro* studies confirm that R5 isolates preferentially infect CCR5+ activated memory T cells, whereas X4 isolates infect CXCR4+ cells, which generally have a naïve or resting memory phenotype [73]. These resting cells may not provide the intracellular requirements for productive infection of X4 viruses [80–82].

An additional mechanism that may influence transmission at mucosal level is mediated by the coreceptor/chemokine system. Mucosal epithelial cells naturally secrete high levels of SDF-1 α , the natural ligand of CXCR4 [83]. Consequently the restriction in transmission of X4 variants may arise from a combination of high level of HIV-1 inhibitory chemokine and a consequent down-regulation of CXCR4 on T-lymphocytes and macrophages in the tissues. This observation may provide an explanation for the restricted transmission of X4 viruses at mucosal level, but not for that *via* the parental route, which occurs despite similar levels of expression of the two co-receptors on cells in the peripheral blood [64,66,68,74,84,85].

The differential use of CCR5 by R5 and R5X4 viruses has also been proposed as a possible explanation for the infrequent transmission of CXCR4-using viruses. Apparently, the capacity of R5X4 viruses to use either co-receptor compromises the interaction with CCR5. Indeed, the β -chemokines preferentially inhibit the dual tropic virus isolates compared to R5 viruses when grown in cell lines expressing only CCR5 [86]. Moreover it has been shown that infection with R5X4 is affected by amino acids substitutions throughout the extracellular domains of CCR5 [87,88], whereas infection with R5 viruses mainly by changes in the N-terminus of CCR5 [89].

Last, an alternative hypothesis is that both R5 and X4 viruses are transmitted, but the latter ones are subsequently sequestered or selectively eliminated [90,91]. Some studies suggested that CXCR4-using viruses isolated during the acute phase of infection were quickly

suppressed in favour of R5 variants through an immune mechanism [92,93]. Although neutralizing antibodies were supposed to be involved, R5 and X4 viruses were shown to have similar sensitivity to neutralization [94–96].

6. Phenotype variation during disease progression

Although R5 variants generally initiate infection and predominate in the early phase of the disease [42,55–61], during the disease course in about one-half of HIV-1 subtype B infected individuals the virus phenotype evolves from CCR5 to CXCR4-usage [97–99]. Two closely related questions are topic of a long lasting discussion. First, why do CXCR4-using viruses appear and dominate only late in infection, and second why is their appearance linked to a more rapid disease progression.

The general immune activation occurring in later stages of infection may result in the proliferation of naïve cells, which are preferentially infected by CXCR4-using variants [100]. Blaak et al. demonstrated that the frequency of HIV-1-infected naïve cells correlated with the rate of overall CD4+ T cell decline [73]. These studies suggest that X4 variants may arise due to a limited number of available CCR5+ target cells or, alternatively, because of the high number of CXCR4+ naïve cell.

For a long time, it has been clear that the emergence of CXCR4-using viral variants is linked to an increased drop in the CD4+ T cell count [97,98] and is predictive of a poor prognosis in adults and children [101–104], suggesting that co-receptor usage strongly influence disease progression. Several mechanisms have been proposed. CXCR4-using viruses were described as more virulent than R5 viruses, with an increased cytopathogenicity and replication rate [24,105–108]. The ability of CXCR4-using viruses to infect thymocytes, the precursor cells of mature CD4+ T lymphocyte, may affect thymopoiesis and account for the accelerated T-cell decline [109]. Immature thymocytes express high levels of CXCR4 but almost no CCR5, and thus can preferentially be infected with CXCR4-using variants [110]. This hypothesis is corroborated by the finding that naïve CD8+ T cells are somewhat reduced in patients with X4 variants as compared to patients with only R5 variants, which may suggest that infection and depletion of the precursor cells occurred [111].

The hypothesis of the “immune-control” to address the co-receptor switch has been recently re-

viewed [112]. In accordance with this hypothesis, X4 viruses are better recognized by the immune system than R5 viruses and, consequently, better suppressed. In a recent study, Mild et al. hypothesized that the frequently observed inpatient recombination of R5 and X4 virus variants, may result in virus variants with the potential to evade the immune system and also to better infect CXCR4-expressing cells [113]. This could easily contribute to the emergence of X4 viruses in the later stages of infection.

It remains to be explained why R5 viruses persist during the entire course of the disease and cause CD4+ T cell depletion and AIDS in the other half of subtype B infected individuals. In addition, the phenotypic switch to CXCR4-usage occurs only in rare cases among subtype C individuals [114,115]. R5 viruses isolated from AIDS patients (“late” R5) displayed an enhanced cytopathic effect compared to R5 viruses isolated from the same individuals at the early stage of disease (“early” R5) [116]. This enhanced cytopathic effect was paralleled by a decreased sensitivity to inhibition by the β -chemokine RANTES [117,118]. In addition these “late” R5 viruses were also more resistant to entry inhibitors and required lower expression levels of CD4/CCR5 [119].

Recently, it was shown that “early” R5 viruses have an R5^{narrow} phenotype, as they are able to exclusively use the *wild-type* CCR5 for entry into target cells. During disease progression, R5^{broad} viruses able to use CCR5/CXCR4 chimeric receptors emerge. The ability to use chimeric receptor was linked to an increased efficiency in CCR5 usage. The evolution from R5^{narrow} to R5^{broad} phenotype was significantly associated with CD4+ T cell decline and with an increased resistance to inhibition by RANTES [29]. Thus, R5 viruses may evolve during the course of the disease towards more efficient CCR5 usage, which confers increased affinity for CCR5, enhancing the CD4-CCR5 interaction. This may favour an increased resistance to inhibition with the natural ligands and possibly an increased ability to infect target cells with low receptor density. In turn, this may result in a larger repertoire of available target cells and thereby increased cytopathogenicity and a more rapid disease progression (Fig. 2).

7. Relevance of co-receptor and chemokine genetic polymorphisms for transmission, replication and pathogenesis

Additional evidences supporting the role of R5 viruses in HIV-1 transmission came from genetic studies an-

Fig. 2. Model of R5 viruses evolution during disease progression. R5 phenotype evolves during the pathogenic process from R5narrow to R5broad. The phenotypic variation is characterized by a more flexible and efficient use of the CCR5 co-receptor, which correlates with CD4+ T cells decline and increased resistance to RANTES inhibition.

analyzing polymorphisms in CCR5 gene. Particularly interesting is a 32-nucleotide deletion ($\Delta 32$) that renders the CCR5 co-receptor non functional [120–122]. Cells from CCR5 $\Delta 32$ homozygotes are highly resistant to *in vitro* infection with R5 variants but permissive for X4 variants or dual tropic strains [12,120,122]. The incidence of the $\Delta 32$ CCR5 allele is high in Caucasian population, approximate 1% are homozygous and 20% heterozygous, but appears only sporadically in Asian and African populations [12].

Studies on large cohorts of individuals exposed to HIV-1 either sexually, parenterally, or *via* mother-to-child (MTCT) transmission, described that the homozygous mutation confers high level of resistance to infection [120,123–127]. However, protection is not absolute and some rare cases of HIV-1 infection despite this genotype have been reported [128–131]. These patients harboured CXCR4-using viruses, suggesting that CXCR4 was the co-receptor responsible for initiating infection.

Individuals heterozygous for the mutation express lower levels of CCR5 [38] but remain susceptible to HIV-1 infection. The frequency of $\Delta 32$ CCR5 heterozygotes in highly exposed but uninfected individuals is similar to that in the seropositive population [123, 124,132–134]. Exceptions have been reported in three studies, two of adults and one of infants, suggesting a reduced risk of transmission inferred by the heterozygous mutation [12,135,136]. However, heterozygous individuals display a slower progression towards

AIDS [123,132,133,137,138], characterized by a slower decline of CD4+ T cells and a lower viral load [139].

A series of studies showed that the same 32-basepair deletion of the CCR5 gene, when detected in the HIV-1 infected mother, does not correlate with transmission [135,136,140–145]. It appears, however, to exert a protective effect against MTCT transmission in those children exposed to a low maternal viral burden of an R5-type isolate [146]. The lack of association between $\Delta 32$ CCR5 heterozygosity and the risk of vertical transmission suggests that HIV-1 could utilize a receptor other than CCR5 to infect cells present at the fetus/infant's mucosal sites. Indeed alternative receptors used by HIV-1 have been identified on mucosal epithelial cells [147–150]. An international meta-analysis study associated the $\Delta 32$ CCR5 polymorphism with a decreased risk of death among perinatally infected children, but only for the first year of life [151].

Additional polymorphisms in the CCR5 gene have been identified, the majority of which are single amino acid substitutions. McDermott et al. identified an A/G polymorphism at basepair 59029 of the CCR5 promoter [152], which was associated with a delayed progression to AIDS in a cohort of HIV-1 seroconvertors lacking both $\Delta 32$ CCR5 and CCR2-641. Conversely, another polymorphism in the CCR5 promoter region, defined CCR5 P1, was shown to accelerate disease progression in homozygous individuals [153]. Finally a mutation (m303) that causes a premature stop codon that prevents the expression of CCR5 appears to con-

fer resistance to an individual carrying the 32-basepair deletion [154]. Additional mutations in the CCR5 gene have been reported, however their influence on HIV-1 *in vivo* is not clear [155–157].

Polymorphisms in receptors other than CCR5 have been identified. A single nucleotide mutation in the CCR2 receptor which results in the substitution of a valine residue for an isoleucine at position 65 (CCR2-64I) has no effect on sexual transmission [158], but exerts a protective effect on MTCT and on disease progression in perinatally infected children [151,159].

A homozygous mutation at position 881 of the 3' untranslated region of the SDF-1 gene (SDF-1 3'A), which encodes the ligand for CXCR4, was shown to protect adults exposed to HIV-1 from infection and to delay disease progression [160]. However, several subsequent studies showed a correlation between the presence of the mutated allele and accelerated progression of HIV-1 infection to AIDS or death [161–164]. With regard to pediatric HIV-1 infection, one study has shown that the mother's, but not the infant's SDF-1 genotype was associated with MTCT [165] and another showed that the protective effect of the heterozygous form of the Δ 32 CCR5 was restricted by the SDF-1 genotype in HIV-1 infected children [166]. We have shown that the presence of the SDF-1 3'A gene correlates with accelerated disease progression in HIV-1 infected children born to seropositive mothers but does not protect against MTCT of HIV-1 [167].

A rapid progression to AIDS was reported in HIV-1 infected individuals with a structural variant of the chemokine receptor CX3CR1, used by HIV-1 as coreceptor in the Central Nervous System [168]. Patients homozygous for CX3CR1-I249 and M280, a variant haplotype affecting two amino acids (isoleucine-249 and methionine-280) progressed to AIDS more rapidly than those with other haplotypes. However, children with the CX3CR1-I249 genotype experienced more rapid disease progression compared to those with the CX3CR1-I249 M280 haplotype [169].

8. Viral phenotype as a marker to predict HIV-1 mother-to-child transmission and pediatric disease progression

Viral genotype and phenotype have given little evidence of a specific pattern associated with MTCT of HIV-1. We and other groups have studied the correlation between co-receptor usage and risk of transmission, and have described that most maternal isolates

are able to use CCR5 as co-receptor, either alone or in association with CXCR4 or other chemokine receptors, independently from transmission (reviewed in [170]). Thus, CCR5 usage is not a useful predictive marker of transmission. Conversely, in an earlier study we showed that mothers who harbour virus with high replicative and SI capacity (R5X4/X4 virus) had a significant higher risk to infect their children than mothers with slow/low (R5) virus [58]. Most vertical transmissions occurred with CCR5-using viruses irrespective of HIV-1 genetic subtype [55,57,58,62].

Recently we took in consideration the possibility that R5^{broad} viruses with a more flexible use of CCR5 co-receptor could influence MTCT of HIV-1. Although the R5^{broad} phenotype was not linked to higher risk of transmission, we demonstrated that the maternal viral phenotype (either R5^{narrow} or R5^{broad}) is generally preserved during transmission and can possibly be predictive of the phenotype of the newborn's viral variant [171]. On the contrary, the R5X4 phenotype was predominantly lost during transmission. Several reports showed that X4 viruses can be transmitted to the newborn, but only in a limited number of cases. In our studies, this low frequency of transmission was closely related to the rare presence of X4 viruses in transmitting mothers [58].

Our data suggested that mothers carrying R5X4 viruses had phenotypically highly heterogeneous populations. We documented the transmission of multiple viral variants with different co-receptor usage in a Δ 32 CCR5 heterozygous child, and demonstrated that the heterozygous genotype *per se* does not contribute to the restriction of R5-type virus spread [172]. It remains to be solved why CXCR4 using viruses are not preferentially maintained during transmission, despite the high prevalence of CXCR4+ naïve T cells in neonates compared to memory CCR5+ CD4+ T cells [173]. Whether a selective process or simply a random event governs transmission remains, however, a topic of discussion [57,97,174,175].

It is intriguing that the early stage of pediatric HIV-1 infection was characterized by an R5^{broad} phenotype, which in adults appeared only at an advanced stage of the disease. This means that infection in children can be established by viral variants with an *env* conformation that allows for a more efficient CCR5 use. In addition, the R5^{broad} phenotype was shown to be a new predictive marker of rapid disease progression and early immunological failure in infected children [171]. R5^{broad} viruses seem to determine detrimental effects similar to those known for CXCR4 using viruses. These data sup-

Table 1
Changes within R5 phenotype over time in two children

Child code	Age (months)
		CCR5	CXCR4	FC-1	FC-2	FC-4b
A	9	+	-	-	-	-
	16	+	-	-	-	-
	40	+	-	-	+	-
	46	+	-	-	+	+
	52	+	-	+	+	+
	67	+	-	-	+	+
B	6	+	-	-	-	-
	13	+	-	-	+	-
	19	+	-	+	+	+
	26	+	-	-	+	+
	34	+	-	-	+	+
	42	+	-	-	-	-

Infection of U87.CD4 cells expressing CCR5, CXCR4 or one of the chimeric receptors FC1, FC2 or FC-4b with sequential virus isolates from two siblings.

+ denotes viruses using the specific coreceptor

port the finding by Casper et al, who suggested that the immunological deterioration in HIV-1 infected children precedes the viral phenotypic switch to CXCR4 usage [101]. We suggest that pre-existing R5^{broad} viruses may have caused the worsening of the disease. Interestingly, in our study all but one newborn's R5^{broad} viruses were capable of a specific chimeric receptor usage (FC-4b), which was previously shown to be linked to evolution to CXCR4 use in adults [29].

Evolution to CXCR4 receptor usage was repeatedly documented in infected children [55,103]. If the appearance of CXCR4-using variants over time is due to an early inhibition of transmitted virus or viral evolution is not defined yet. Phylogenetic studies of two mother-child pairs revealed that the appearing X4 virus in the child was due to viral evolution from their own R5 population rather than caused by transmission of the mother's X4 strain [176].

We have recently reported that viral isolates of infected children display an R5 phenotypic evolution during disease progression [177]. The children were infected with HIV-1 of R5 phenotype, which persisted throughout follow-up, but evolved to better utilize CCR5 over time (Table 1). This phenotypic evolution from *wild-type* R5 to broad chimeric receptor using viruses was also characterized by an increase of RANTES resistance *in vitro* [177]. Our hypothesis is that the reduced

sensitivity to RANTES inhibition of R5^{broad} viruses could become the force driving the viral evolution to CXCR4 usage. The different sensitivity of R5^{broad} viruses to RANTES should be investigated in the view of treatments with small CCR5 entry inhibitors.

9. Concluding remarks

Since the discovery of chemokine receptors as HIV-1 co-receptors, promising new strategies for the development of anti-HIV-1 agents and vaccines have been envisaged. One of the most promising approaches has been to interfere with HIV-1 co-receptor binding with chemokines-derivatives/analogues. As individuals with the $\Delta 32$ homozygote condition appear to be healthy, and the CCR5 function redundant, several compounds directed against CCR5 have been developed in the last years, including covalently modified natural CCR5 ligands, antibodies and small-molecule antagonists. One of these inhibitors (maraviroc, Pfizer, Inc.) has been approved for clinical use, a second (vicriviroc, Schering-Plough Research Institute) is in phase 3 trials, and several others are in preclinical or clinical development (for review see [178]). However it is not clear whether such antagonistic agents could favour the emergence of the more pathogenic CXCR4-

using viruses or variants that exploit alternative co-receptors. Thus, HIV-1 phenotyping becomes mandatory with the introduction of these drugs. However, the principal resistance pathway to CCR5 antagonists was shown *in vitro* to involve CCR5 usage in an inhibitor resistant manner [179], without switch to CXCR4 usage. If the sub-classification of R5 viruses in narrow and broad could explain this resistance *in vivo*, has still to be investigated.

Small positively charged peptides have also been reported that interact with CXCR4 and block infection of X4 strains [180,181]. Among CXCR4 antagonists, AMD070 is currently the unique molecule in clinical development and phase 1b/2a clinical trials are ongoing [182].

A recently described successful transplantation of allogeneic stem cells homozygous for the CCR5 delta32 allele to a patient with HIV, who subsequently controlled the infection in absence of antiretroviral therapy supports further development of CCR5-directed drugs or vaccines [183].

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