HIV-1 receptors and cell tropism

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HIV virus particles interact with several receptors on cell surfaces. Two receptors, CD4 and a co-receptor act sequentially to trigger fusion of viral and cellular membranes and confer virus entry into cells. For HIV-1, the chemokine receptor CCR5 is the predominant co-receptor exploited for transmission and replication *in vivo*. Variants that switch to use CXCR4 and perhaps other co-receptors evolve in some infected individuals and have altered tropism and pathogenic properties. Other cell surface receptors including mannose binding protein on macrophages and DC-SIGN on dendritic cells also interact with gp120 on virus particles but do not actively promote fusion and virus entry. These receptors may tether virus particles to cells enabling interactions with suboptimal concentrations of CD4 and/or co-receptors. Alternatively such receptors may transport cell surface trapped virions into lymph nodes before transmitting them to susceptible cells. Therapeutic strategies that prevent HIV from interacting with receptors are currently being developed. This review describes how the interaction and use of different cellular receptors influences HIV tropism and pathogenesis *in vivo*.

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The main cells targeted by HIV *in vivo* are T-cells, macrophages and probably dendritic cells. This narrow tropism is predominantly determined by the cell surface receptors required for HIV to attach to and gain entry into cells. Two different receptors, CD4 and a co-receptor, are usually essential for HIV to infect cells efficiently. The chemokine receptor CCR5 is the co-receptor predominantly used *in vivo*; however, variants that use another co-receptor, CXCR4, evolve during disease in some AIDS patients. *In vitro*, more than a dozen different co-receptors have been identified that support infection of cell lines by different HIV strains. The capacity to exploit alternative co-receptors ought to be advantageous and confer a wider cell tropism; however, current evidence suggests that co-receptors other than CCR5 or CXCR4 have limited use *in vivo*. The factors that preclude the use of a wider range of co-receptors *in vivo* are not known, nor is it clear why such a variable virus as HIV fails to evolve variants capable of exploiting

alternative co-receptors and colonizing a broader range of cell types. This article will discuss the cell types infected by HIV and how the use of different receptors influences cell tropism and pathogenesis *in vivo*.

Cell surface receptors for HIV entry into cells

HIV interacts with CD4 and a seven transmembrane (7TM) co-receptor to trigger entry into cells. The envelope glycoprotein spikes on the surface of virus particles comprise an outer surface gp120 (SU) non-covalently linked to a transmembrane gp41 (TM). Each spike on the virus particle comprises a trimer of three gp120 and three gp41 molecules. Binding of CD4 to gp120 triggers a structural change, which exposes a binding site for a co-receptor (Fig. 1). Further structural re-arrangements are initiated when the co-receptor is bound. These changes occur predominantly in gp41 and are thought to be sufficient to trigger fusion of viral and cellular membranes and entry of the virion core into the cell's cytoplasm. In the absence of CD4, infection is

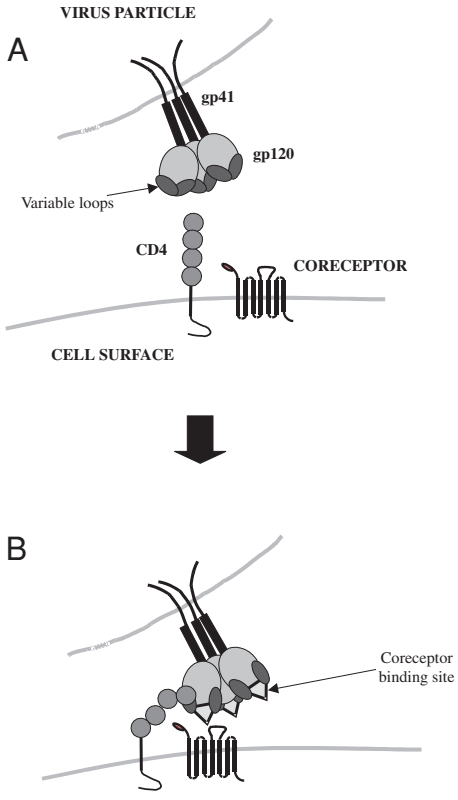

Fig. 1 Receptor interactions involved in HIV entry. (A) HIV virion binds CD4. (B) CD4 binding induces conformation changes in gp120 that result in the movement of the variable loops and exposure of the co-receptor binding site. Flexible regions in CD4 between domains 2 and 3 as well as between domain 4 and the membrane allow orientation of the co-receptor binding site for co-receptor binding.

inefficient and its significance *in vivo*, controversial. Over 14 different 7TM receptors have been identified as potential co-receptors for HIV and SIV by their capacity to support infection of CD4+ cell lines *in vitro* (Table 1). These receptors are members of (or closely related to) the chemokine receptor family. CCR5 and CXCR4 are the major co-receptors and all HIV-1 isolates can use one or both. Several polymorphisms in the CCR5 gene that influence HIV transmission and/or disease progression have highlighted the importance of this co-receptor *in vivo*[1]. The most significant polymorphism is the 32 base pair deletion (Δ32 CCR5) in the coding region that results in a defective CCR5 product that fails to reach the cell surface[2]. Homozygotes are, therefore, effectively CCR5-negative. Their substantial resistance to HIV infection whether the risk to infection is via sex, blood contact[3] or from mother-to-child[4] clearly illustrates a major role for CCR5 during transmission. CCR5, however, is not the only transmission route and a few HIV+ Δ32 CCR5 homozygotes have been identified. These individuals (where tested) appear to carry CXCR4-using viruses[5]. The defective Δ32 CCR5 gene product can still form oligomers with wild-type CCR5 in the endoplasmic reticulum and thus heterozygotes are likely to lose more than 50% of cell surface CCR5[2]. HIV+ Δ32 CCR5 heterozygous individuals suffer a significantly slower disease course demonstrating the importance for CCR5 in HIV pathogenesis. No significant CXCR4 polymorphisms have been reported probably because CXCR4 is an essential requirement in development and the 'knockout' phenotype in mice is lethal. The faster disease progression and rapid loss of CD4+ T-cells associated with the emergence of CXCR4-using viruses indicate an important *in vivo* role for CXCR4 in some individuals[6]. The

Table 1 Co-receptors that function for HIV and SIV on CD4+ cell lines. Only CCR5 and CXCR4 have so far been shown to function as co-receptors *in vivo*

Co-receptors	Ligands	Role for viral replication	
		In vitro	*In vivo*
CCR1	MIP-1α, RANTES, MPIF-1, MCP-3	+	
CCR2b	MCP-1, MCP-2, MCP-3	+	
CCR3	Eotaxin, Eotaxin-2, MCP-3, MCP-4, RANTES	++	
CCR5	MIP-1α, MIP-1β, RANTES, MCP-2	++++	++++
CCR8	I-309	+	
CCR9	TECK	+	
CXCR4	SDF-1	+++	++
CX3CR1/V28	Fractalkine	+	
STRL-33/BONZO/CXCR6	CXCL16	+	
GPR1	?	+	
GPR15/BOB	?	+	
APJ	Apelin	+	
ChemR23	?	+	
RDC1	?	+	
Leukotriene B_4 receptor	Leukotriene B_4	+	

significance of other co-receptors for HIV-1 replication *in vivo* and pathogenesis remains unclear. Recent evidence, however, has demonstrated that STRL-33 (now termed CXCR6) is associated with infection of a subset of primary T-cells *in vitro*[7], while CCR8 supports infection of thymocytes[8]. The capacity of these co-receptors to support infection of such primary cell cultures (rather than indicator cell lines where recombinant CD4 and co-receptors are often expressed at unnaturally high levels) provides stronger support of a possible role *in vivo*.

The interaction between gp120 and co-receptors

The interaction between CD4 and gp120 is conserved among all primate lentiviruses. Some of the amino acids on gp120 that form the CD4 binding site are variable, however, for these residues, the peptide backbone rather than their side chains are involved in contacting CD4[9]. The co-receptor binding site on gp120 is not usually fully exposed until CD4 is bound. The variable V1/V2 loops are probably the main cover for the co-receptor binding site and these loops become repositioned when CD4 is bound[9]. Mutations that expose the co-receptor binding site, therefore, confer a more CD4-independent phenotype[10,11].

The regions of gp120 implicated in the interaction with co-receptors are thought to involve the relatively conserved 'bridging sheet' that lies between the protruding and variable V1/V2 and V3 loops, as well as some amino acids in V3 itself[9]. The V3 loop has long been known to be a major determinant of cell tropism and now co-receptor use. Positively charged amino acids in V3 that confer a syncytium inducing (SI) phenotype correlate with CXCR4 use. The role of the V1/V2 loops in the co-receptor interaction is less clear since an HIV-1 mutant with V1/V2 deleted was infectious[12], while recombinant gp120 similarly deleted for V1 and V2 also bound co-receptors[13]. When present, however, V1 and V2 influence both cell tropism and co-receptors used[14].

Chemokine receptors form rods in the cell membrane with a central pore surrounded by the seven transmembrane regions. Four domains are exposed on the cell surface, the N-terminus, and three extracellular loops (E1, E2 and E3). Co-receptors take up different conformations on cell surfaces and on different cell types[15] that influence their ability to support HIV infection. Such conformations may result from the formation of dimers[16] or association with other cell surface molecules as reported for CCR5 and CD4[17]. Two sites on co-receptors centred around the N-terminus and E2 are involved in HIV entry. Mutagenesis studies showed the N-terminal domain of CCR5 is important for co-receptor activity for CCR5-using (R5) HIV-1s[18]. R5 strains, however, differ considerably in their use of CCR5 as highlighted by a wide variation in their capacity to

infect cells expressing different chimeric human/mouse CCR5s[19]. For SIV_{MAC}, both macrophage-tropic and T-cell tropic strains use CCR5; however, the former require the N-terminus of CCR5, while E2 is crucial for T-tropic SIVs[20]. It is unclear if there are HIV-1 CCR5-using strains with the properties of T-tropic SIVs.

For CXCR4-using (X4) strains, E2 is critical and deletion of the N-terminus of CXCR4 has little affect on some but not all strains[21,22]. Chimeric co-receptors, therefore, support X4 virus entry as long as E2 of CXCR4 is present; however, Brelot *et al*[23] showed that X4 strains vary in their use of CXCR4 E2 with different isolates dependent on distinct E2 residues for activity.

Electrostatic charge interactions are also involved and likely to enhance gp120/co-receptor interactions. The N-terminal region of CCR5 (and often other co-receptors) is negatively charged due to 3 acidic amino acids and 4 (potentially) sulphated tyrosine residues which are important for co-receptor function[24]. These negative residues may aid interactions with positive amino acids in and around the bridging sheet on gp120[9]. Moreover, the V3 loops of X4 strains are highly positively charged while E2 of CXCR4 contains five negatively charged amino acids and it is likely that these oppositely charged faces interact. Mutagenesis of all five acidic residues, however, does not completely eliminate HIV infection[25]. Thus, negatively charged residues at the N-terminus of CCR5 and in E2 of CXCR4 may enhance the gp120/coreceptor interaction by electrostatic interactions with R5 and X4 strains respectively, however, they do not determine the specificity of the interaction.

Sites in the V1/V2 loop, the bridging sheet and V3 loop on gp120 may thus contribute to at least two specific interactions with co-receptors centred on the N-terminus and E2. A 'high affinity' interaction at both sites may not be needed to trigger infection, explaining why the specificity of the co-receptor interaction can be predominantly mapped to either the N-terminus or to E2. In summary, diverse virus strains vary considerably in the regions and specific amino acids of co-receptors that they exploit for recognition and triggering fusion. The capacity of HIV to vary the envelope and co-receptor residues involved in their interaction will be a major mechanism of immune evasion.

Cell tropism of HIV in immune and non-immune tissues

In vivo, HIV mainly infects haematapoietic cells that express CD4 and either CCR5 or CXCR4. These include T-helper lymphocytes, macrophages and probably dendritic cells. Many reports describe the infection of CD4-negative non-haematapoietic cell types *in vitro*.

Generally this type of infection is inefficient and its significance for pathogenesis and viral reservoirs *in vivo* remains controversial. *In vitro*, R5 viruses infect primary cultures of both lymphocytes and macrophages, while X4 isolates also infect T-cell lines. The capacity of X4 strains to infect macrophages is controversial[26]; however, we and others have shown that primary X4 isolates infect at least some populations of macrophages[27]. In the blood of individuals that carry R5 viruses, the $CD4^+$ $CD45RO^+$ memory T-cells carry most of the proviral load, although $CD45RA^+$ naïve cells are also infected. When CXCR4-using strains emerge, their tropism for different T-cell populations is broader. On T-cells, CCR5 expression is mainly restricted to memory cells, while CXCR4 expression is more widespread but predominates on naïve T-cells[28]. Symptomatic, X4-carrying individuals thus have an increased proviral load in naïve T-cells consistent with an expanded T-cell tropism[29]. Early studies suggested that monocytes were infrequently colonized *in vivo*[30]; however, more recent reports indicate that monocytes may harbour replication competent virus in patients treated by HAART[31,32]. Whether dendritic cells are infected has been controversial; however, the current consensus suggests that they are likely to support at least some level of HIV replication *in vivo*, and may play a significant role in transferring newly transmitted virus from mucosa to T-cells in lymph nodes[33]. Suggestions that the capacity to replicate in dendritic cells reflected a mucosal route of transmission and was dependent on HIV-1 subtype[34] have been refuted by others[35]. The extent dendritic cells support full replication may depend on their state of maturation[36]. Immature dendritic cells were reported to selectively support replication by R5 viruses[37], while more mature cells are permissive to R5 and X4 virus entry, but less supportive of post-entry events[37]. Dendritic cells can also trap virus particles by high affinity interactions between sugar groups on gp120 and lectin-like domains on the receptor DC-SIGN[38]. DC-SIGN may, therefore, enable dendritic cells to trap HIV particles and pass them to T-cells while also presenting antigen. *In vitro*, conjugates of T-cells and purified dendritic cells provide a rich environment for intensive replication and production of new viral particles and may also trigger full replication in cells harbouring a restricted infection. Immature dendritic cells, *e.g.* Langerhans' cells at mucosal membranes, are likely to be the first cells encountered by HIV following transmission. These cells may carry HIV either as by DC-SIGN-trapped virus or as an infected cell to lymph nodes where association with T-cells provides a potent medium for rapid amplification of virus.

Chemokines also influence the types of cells that become infected. Saha *et al* showed that several $CD4^+$ $CCR5^+$ T-cell clones derived from non-progressing $HIV\text{-}1^+$ individuals and transformed by Herpes Saimiri virus (HSV) were resistant to infection by R5 HIV-1 strains due to the production of endogeneous β-chemokines[39]. Saha's study also showed that T-cell clones made from patients who had advanced to AIDS were

substantially more sensitive to R5 virus replication consistent with the increasing sensitivity and colonization of $CD4^+$ $CCR5^+$ T-cells as disease progresses. Extensive expression of SDF-1 along mucosal membranes has been detected as well as down-regulation of CXCR4 on T-lymphocytes in the vicinity[40]. These observations suggest a mechanism for the blocked transmission of X4 viruses across mucosal membranes and may explain why dendritic cells *in vitro* and away from the SDF-1 rich environment of mucosa[40] support at least the early entry stages of X4 viruses. Other mechanisms, however, are required to explain the selective transmission of R5 viruses directly into the blood. Thus, soluble factors, *e.g.* chemokines in the tissue milieu or produced endogenously by target cells, can have a major influence on tropism.

In non-immune tissues and organs, the resident specialized macrophage cells carry the viral load. For example, HIV antigens can be detected in the liver macrophages known as Küppfer cells, while alveolar macrophages are infected in the lung. The brain is physically isolated from the blood by the blood–brain barrier, a system of tight gap junctions between endothelial cells in the blood capillaries. The brain is colonized by HIV-1 early in infection and probably seeded by HIV carried in by infected monocytes or macrophages. The main cell types infected in the brain are perivascular macrophages and microglia[41]. Non-haematapoietic astrocytes that do not express CCR5 or CD4 may also become infected, but do not efficiently support production of new virus particles[42]. The extent of astrocyte infection and its significance for brain pathology is controversial. Infection of brain microvascular endothelial cells (BMVECs) is even more contentious but supported in some studies[43], and would represent a simple route of entry into the brain across the blood–brain barrier.

Variation of co-receptor use *in vivo*

The extent HIV-1 adapts to replicate in different cell types or to exploit co-receptors other than CCR5 or CXCR4 *in vivo* is not known. The growing number of different 7TM receptors that support HIV and SIV infection of cell lines *in vitro* does not accurately predict co-receptor usage *in vivo*. High level expression of alternative co-receptors 'out of context' on cell lines seems to deliver them to the cell surface in an active form that can confer virus entry. Additional factors *in vivo* that may prevent many of the same alternative co-receptors from functioning are not known. Nor is it known what factors and/or selective pressures operate *in vivo* that prevent CXCR4-using (SI) strains from emerging until late in disease and often not at all. Both immune (*e.g.* neutralizing antibodies) and non-immune (*e.g.* SDF-1 blockade and/or down-regulation of CXCR4) mechanisms have been suggested to contribute

(reviewed by Michael and Moore[44]). The R5 to X4 switch usually occurs via an evolution through an R5X4 stage[6]. The capacity to exploit both CCR5 and CXCR4, however, compromises the interaction with CCR5 and such strains are often ultrasensitive to inhibition by β-chemokines[45]. This reduced CCR5 interaction probably explains why R5X4 viruses cannot follow the CCR5 route for transmission and like SI strains in general are transmitted infrequently.

The evolution of R5 to R5X4 and X4 strains in about 50% of symptomatic individuals, however, illustrates the capacity of HIV-1 to switch co-receptors *in vivo*. Data from SIVs also show the potential of primate lentiviruses to adapt to use alternative co-receptors. For instance, an SIV that predominantly uses CCR2b is present in red capped mangabeys that carry defective CCR5 genes[46]. Alternative unidentified co-receptors are frequently found to support HIV-2 and SIV infection of primary T-cells and macrophages *in vitro*[47] and, as already discussed, evidence is now emerging that implicates CXCR6 (STRL-33/BONZO) for HIV-1 infection of a T-cell subset and CCR8 for thymocytes[7,8].

Co-receptor switching (analogous to an R5 to X4 switch for HIV-1) has not been demonstrated *in vivo* for SIV, although an evolution from macrophage-tropism (M-tropic) to T-cell tropism (T-tropic) has been implicated[48], while neurotropic and neurovirulent SIV_{MAC} strains that rapidly cause brain disease have also been isolated[49]. The switch from M- to T-tropism involves a change in how CCR5 is exploited as a co-receptor rather than the use of an alternative co-receptor. Thus, two potential pathways for envelope evolution exist *in vivo*; one involves a switch to a new co-receptor, while the second involves a change in how a particular co-receptor is exploited to trigger infection.

Are there HIV-1 variants tropic for specialized cells in different tissues?

It is not known if specific variants evolve that have an increased capacity to infect and replicate in the specialized cells of different tissues or if such variants are linked with particular AIDS pathologies, *e.g.* dementia. As already discussed, neurovirulent SIV_{MAC} variants can be isolated and their properties are conferred by determinants that include sequences in the envelope gene[50]. This precedent demonstrates the real possibility that similar neurotropic HIV-1 strains exist that are associated with dementia. Moreover, specific amino acids at particular sites in the V3 loop (or motifs) have also been associated with envelopes in the brain[51,52]. Such motifs are highly controversial, but could be associated with the use of alternative brain encoded co-receptors or adaptation to use CCR5 conformations expressed on brain cells. To date, all brain-derived viruses, support the predominant use of CCR5 in brain tissue[53]. The possibility that viruses in

the brain broaden their co-receptor usage from R5 to include an unknown co-receptor expressed on specialized brain cells has not been excluded. Furthermore, most SIV_{MAC} strains use several co-receptors including CCR5, STRL-33, GPR1 and GPR15, thus raising the possibility that one or more are preferentially used to infect specialized cells in different tissues. Further investigation of the tropisms and co-receptors used by envelopes present in the brain and other tissues is clearly needed.

HIV variation in different tissues

Independent HIV variation in different body compartments has been well documented, *e.g.* in the brain[51,54] and in semen[55,56]. This variation is distinct from that seen in blood or lymphatic organs and may represent selection for tissue-adapted variants, or just random but independent evolution. Regardless, variation in the envelope will help the virus to escape from neutralizing antibodies; however, too much divergence is constrained since it will weaken the envelope's interactions with CD4 and co-receptors, reducing the efficiency of infection and probably increasing sensitivity to inhibition by chemokines. Selection pressures in different compartments will vary greatly. For example, the brain is a relatively 'immunoprivileged' environment and viruses replicating there will not be exposed to the same constraints imposed by neutralizing antibodies in lymphoid tissue. Viral strains in the brain may, therefore, adopt a more 'open' envelope conformation that allows enhanced interactions with CD4 and co-receptors, as seems to occur with T-cell line adapted (TCLA) strains that have been cultured *in vitro* in the absence of neutralizing antibodies[57]. Moreover, concentrations of inhibitory chemokines are likely to vary considerably depending on the tissue and levels of cellular activation. CCR5 may also be expressed in distinct tissue or cell type specific conformations that support infection of some R5 variants over others, as we reported for particular R5 viruses that failed to enter primary $CCR5^+$ macrophages[58].

Envelope/co-receptor interactions may also influence early post-entry events[59] in some cell types favouring some strains over others. For instance, the observations that both M-tropic and T-tropic SIV_{MAC} strains enter macrophages[60], while only M-tropic envelopes signal *via* CCR5[61] has raised the possibility that co-receptor signalling events induced by a virus entering at the cell surface may be a requirement for replication in some cell types. Signalling during virus entry, however, is controversial and recent data showing that increased expression of CCR5 on the surface of macrophages fully rescues T-tropic SIV replication probably argue against, in this instance[62].

Thus different cell types in distinct environments will select for or against particular R5 viruses or quasispecies; however, the extent this happens and its impact on pathogenesis is unclear.

The role of other receptors

HIV viral particles interact with a range of other cell surface receptors *via* interactions that involve gp120. These interactions do not actively support HIV entry but aid attachment of HIV virions to cell surfaces that contain suboptimal levels of CD4 or co-receptors, *e.g.* on macrophages and astrocytes. Some of these interactions are mediated by the sugar groups on the envelope glycoprotein associating with other sugars or with receptors that contain lectin-like domains on the cell surface, *e.g.* the mannose specific macrophage endocytosis receptor[63] and DC-SIGN (see above)[38]. HIV envelope gp120 also binds the glycolipid, galactocerebroside (gal-C) and its sulphated derivative, sulphatide[64,65]. These molecules are expressed on neuronal and glial cells in the brain[64], colon epithelial cell lines[65] and importantly also on macrophages[66]. Both DC-SIGN and Gal-C bind gp120 with a high affinity (Kd of 11.6 nM), similar to the binding affinity of monomeric gp120 for CD4 (Kd of 2–5 nM). Gal-C supports suboptimal entry of particular HIV-1 strains without CD4, although infection of the colorectal cell line HT29 requires both gal-C and CXCR4[67]. Mondor *et al*[68] have shown that HIV virions attach to the surface of HeLa/CD4 cells *via* an interaction between gp120 and the glycosaminoglycan moiety (heparan sulphate) on the cell surface. This interaction can be demonstrated for X4 and R5X4 but is less strong for R5 envelopes since it is mediated mainly by positively charged V3 loops interacting with negatively charged sulphate groups on glycosaminoglycans[69]. Although these receptors may aid HIV attachment, fusion will not occur until sufficient CD4 and co-receptor molecules are recruited to trigger formation of a fusion pore. Thus direct and early interactions with CD4 are likely to lead to the most efficient infection process with the fastest kinetics.

Therapies targeted at HIV receptors

Highly active anti-retroviral treatment (HAART) has been very effective in many HIV^+ individuals in reducing viral load and often resulting in dramatic recovery from disease. There is still a need to develop new approaches to therapy that will provide alternative drugs when resistant virus variants emerge or particular drugs are not well tolerated. Many novel strategies that interfere with the entry pathway are being developed. Intervention of the interaction between CD4 and the HIV

envelope is an attractive therapeutic approach since all HIV and SIV strains can bind CD4, while infection without CD4 is probably insignificant *in vivo*. A soluble form of CD4 containing the four extracellular domains was shown to be an excellent inhibitor of infection by TCLA HIV-1 strains[70]. The sensitivity of TCLA viruses was probably due to the capacity of sCD4 to tear gp120 molecules off the surface off virus particles[71]. Sadly, it turned out that primary isolates of HIV-1 (R5 or X4) were substantially more resistant to sCD4 inhibition[72], because they had a lower affinity for CD4 and gp120 was more stably attached to virions[73]. Clinical trials showed that sCD4 was not toxic and well tolerated, but failed to have a major influence on viral load or the decline in CD4 cell numbers in peripheral blood[74,75] except at very high doses[76]. Chimeric CD4 and immunoglobulin (immunoadhesin) molecules consisting of the N-terminal 2 domains of CD4 joined to the Fc region of an antibody (human IgG_1) substantially increased the half life *in vivo* and also conferred antibody functions. CD4-IgG prevented infection of chimpanzees by the prototype TCLA HIV-1 IIIB strain[77]; however, in clinical trials, it had little effect on viral load and declining CD4 cell numbers[78]. Chimeric CD4-*Pseudomonas* exotoxin (CD4-PE40) constructs were also excellent inhibitors i*n vitro,* targeting and killing cells infected with patient isolates that resisted sCD4 neutralization[79]. In clinical trials, CD4-PE40 was too toxic to be used at concentrations effective against HIV[80]. Faced with such failure in the clinic, the drive to develop CD4-based therapies has waned. One surviving approach is a version of CD4-IgG, where the Fv portions of both heavy and light chains have been replaced with D1D2 of CD4. This construct, a heterotetramer of CD4 D1D2, is effective against diverse primary HIV-1 strains[81] as well as plasma virus taken straight from patients (*ex vivo*)[82]. Clinical trials with this CD4-IgG have not yet been reported. New strategies will come from the reported crystal structure of gp120/CD4 complexes[9]. For instance, a cavity at the surface of gp120 was revealed that accommodates the phenyl ring of F43 on CD4. Agents designed to block this cavity would be predicted to interfere with the interaction between gp120 and CD4 and so block infection.

The identification of HIV co-receptors has provided an exciting new therapeutic opportunity. Drugs aimed at blocking envelope interactions with both CCR5 and CXCR4 are being developed. CCR5 is an excellent target for therapy since individuals homozygous for the 32 base pair deletion in CCR5 are effectively CCR5-negative but healthy. Agents that specifically block the natural CCR5 receptor activity should, therefore (at least in theory), not be harmful. There has been much debate about whether inhibitors of R5 strains will select for the more pathogenic X4 variants[44], or for variants that exploit alternative co-receptors. Extensive evidence that shows Δ32 CCR5 heterozygotes progress more slowly to AIDS bodes well for CCR5 inhibitors that will also decrease the level of

functional CCR5 for HIV infection. One report, however, suggested caution and showed that CXCR4-using viruses may be present more frequently in Δ32 CCR5 heterozygotes[83] Moreover, variation in use of CCR5 by different R5 strains[19] may mean that variant viruses will emerge that escape CCR5 inhibitors but still use CCR5 as a co-receptor. Regardless, co-receptor drugs will be used in combination with agents that target other events in the virus life cycle, *e.g.* RT or protease inhibitors. In these situations, virus replication should be driven down to very low levels minimizing the chances of accruing mutations that confer escape from CCR5 inhibitors.

In the early days after co-receptors were discovered, it was hoped that the chemokines themselves or their antagonist derivatives might be exploited in therapy. We reported that a recombinant form of RANTES modified at the N-terminus (amino-oxy-pentane-RANTES, or AOP-RANTES) potently inhibited infection by R5 strains of HIV[84]. The potency of AOP-RANTES was due to its capacity to induce CCR5 internalization and retention in endosomes, a property that effectively removed CCR5 from the cell surface[85]. Small positively charged peptides have also been reported that interact with CXCR4 and block infection of X4 strains of HIV[86,87]. It is unlikely that such peptides or other protein-based drugs can be widely used for treatment of infected individuals since they are costly to manufacture and are likely to require intravenous administration. One great hope for drug therapies that target co-receptors lies in small organic molecules that are less expensive to synthesize and can be taken orally. The optimism comes from past successes in targeting 7TM, GPCRs, where small organic molecules specific for particular 7TMs have been exploited to treat a range of diseases including schizophrenia and asthma. Many pharmaceutical companies hold large collections or libraries of small organic molecules that are currently being screened for activity to CCR5 or CXCR4. Once molecules that interact with CCR5 or CXCR4 have been identified, then further manipulations of the structure can increase specificity, affinity and other properties. Already an antagonist of CCR5 has been reported (TAK-779) that inhibits R5 strains HIV *in vitro*[88], while AMD3100, a bicyclam derivative, binds CXCR4 and blocks X4 viruses[89]. It is certain that several more are currently the subject of patent applications but will be in clinical trials soon. Whether such molecules will be successful in the treating HIV$^+$ patients will become clear within only a few years.

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