Cell surface receptors, virus entry and tropism of primate lentiviruses

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Human immunodeficiency virus (HIV) exploits cell surface receptors to attach to and gain entry into cells. The HIV envelope spike glycoprotein on the surface of virus particles binds both CD4 and a seven-transmembrane coreceptor. These interactions trigger conformational changes in the envelope spike that induce fusion of viral and cellular membranes and entry of the viral core into the cell cytoplasm. Other cell surface receptors also interact with gp120 and aid attachment of virus particles. This review describes these receptors, their roles in HIV entry and their influence on cell tropism.

Introduction

Human immunodeficiency virus (HIV) and the simian immunodeficiency virus (SIV) counterparts are retroviruses that belong to the family of lentiviruses that causes degenerative diseases. A hallmark of lentivirus disease is the failure of the host’s immunity to prevent continuous virus replication. Lentiviruses include maedi–visna virus of sheep, equine infectious anaemia virus and feline immunodeficiency virus (FIV) as well as HIV and SIV. All these lentiviruses establish persistent infections in cells of the monocyte/macrophage lineage that are associated with pathology in the brain (dementia) and joints (arthritis). FIV, HIV and SIV also infect T-lymphocytes and cause severe immune deficiency. For HIV, the cell surface receptors exploited for entry into cells are major determinants of cell tropism and pathology.

Lentiviruses are enveloped viruses that acquire a lipid membrane while budding from the membranes of an infected cell. After budding, the Gag proteins of the virus core are processed by the virion protease to form a mature infectious particle. The resulting cone-shaped core contains the viral genomic RNA that is delivered into a new cell to start a fresh cycle of replication. The first events that initiate infection are: (1) attachment of the virus particle to the cell surface and (2) fusion of the virus and cell membranes to deliver the virion core into the cell cytoplasm (Fig. 1A). For HIV, attachment and fusion are mediated by the interaction of virion glycoprotein spikes with cell surface receptors.

The glycoproteins of many enveloped viruses carry a hydrophobic fusion domain, which is held inside the native spike glycoprotein for protection from hydrophilic environments. Fusion is triggered by conformational changes in the envelope spikes directing the fusion domain to embed in the cell membrane. For example, following attachment to cell surfaces, influenza virus particles are internalized into endosomes. The low pH (< 5.5) inside endosomes triggers reorientation of the influenza virus spike glycoproteins (haemagglutinins, HAs), exposing the fusion domain to the endosomal membrane and initiating fusion (Fig. 1B) (reviewed by Skehel et al., 1995). HIV uses a different strategy to trigger the conformational changes needed for infection. This process is independent of low pH, being driven by interactions with specific receptors on the cell surface. Two receptors are usually essential for HIV entry: CD4 and a seven-transmembrane (7TM) coreceptor (Fig. 2). This review examines these and other cellular receptors that HIV exploits to attach to and gain entry into cells.

HIV envelope structure and attachment of HIV particles to cell surfaces

Attachment of HIV particles to cell surfaces is mainly attributed to the interaction of the spike glycoproteins with receptors. The envelope glycoproteins of HIV are made as a
precursor molecule, gp160, which is cleaved in the Golgi apparatus by a cellular protease (for example, furin and related proteases) (Hallenberger et al., 1997) into a surface (SU) gp120 molecule, noncovalently attached to a transmembrane (TM) gp41. Each spike on a virus particle is made up of three gp120 and three gp41 molecules, held together as a trimer (Weiss et al., 1990) by determinants in gp41 (Earl et al., 1990). The SU glycoprotein gp120 is particularly exposed to host antibodies and contains five variable loops (V1 to V5) which may help replicating viruses to escape antibody-mediated neutralization. These variable loops are interspersed by more conserved regions. In comparison, gp41 is relatively conserved.

CD4 is the major receptor for HIV and SIV (Sattentau et al., 1988). Each monomer of gp120 contains a binding site for CD4. Engagement of one CD4 molecule by a single gp120 in the trimeric spike is sufficient to induce conformational changes in all three glycoprotein monomers of the trimer (Salzwedel & Berger, 2000). HIV type 1 (HIV-1) strains adapted for replication in CD4+ T-cell lines (T-cell line-adapted, TCLA) have an affinity for CD4 up to 50 times higher than envelopes of primary isolates (Moore et al., 1992). Despite CD4 affinities that are often low, primary viruses are still dependent on CD4 for fusion; however, the importance of CD4 for attachment to cells is questionable. Furthermore, while some cell types targeted by HIV in vivo express high levels of CD4 (for example, T-cells), others, including macrophages and dendritic cells (DCs), express barely detectable amounts. In these situations, HIV may attach to cells by CD4-independent interactions involving sugar groups on the envelope glycoprotein with other sugars or lectin-like domains on cell surface receptors, such as the mannose-specific macrophage endocytosis receptor (Larkin et al., 1989). Table 1 lists cell surface molecules identified to interact with gp120. A cell surface protein (DC-SIGN) identified by its capacity to bind gp120 with high affinity (Curtis et al., 1992) is expressed on certain DC populations (Geijtenbeek et al., 2000). A closely related receptor (DC-SIGNR) expressed on endothelial cells binds HIV in a similar manner (Pohlmann et al., 2001). Gp120 also binds the glycolipid galactocerebroside (Gal-C) and its sulphated derivative, sulphatide (Fantini et al., 1993; Harouse et al., 1991). These molecules are expressed on neurons and glia in the brain (Harouse et al., 1991), colon epithelial cell lines (Fantini et al., 1993) and, importantly, on macrophages (Seddiki et al., 1994). Gal-C binds gp120 with a high affinity, similar to
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Fig. 2. Events in the attachment and fusion of HIV. (A) HIV virion binds CD4. (B) Conformation changes in the core of gp120 and movement of the variable loops cause exposure or formation of the coreceptor-binding site. Flexible regions in CD4 between domains D2 and D3 as well as domain D4 and the membrane allow orientation of the coreceptor-binding site for coreceptor binding. (C) Binding of gp120 to the coreceptor triggers further conformational changes, mainly in gp41, which trigger release of the fusion domain and result in it embedding in the host cell membrane. (D) Complexing of the leucine zipper domain (red) and α-helix (blue) repositions the gp41 TM region and fusion domains close together allowing a fusion pore to form (also see Fig. 3).

the binding affinity of monomeric gp120 for CD4. Gal-C supports suboptimal entry of particular HIV-1 strains without CD4, although infection requires a coreceptor (Delezay et al., 1997). Mondor et al. (1998) have shown that HIV virions attach to HeLa cells via an interaction between gp120 and the glycosaminoglycan heparan sulphate. This interaction can be demonstrated for X4 and R5X4 viruses but is less efficient for R5 virus envelopes, since it is mediated mainly by positively charged V3 loops interacting with negative sulphate groups on glycosaminoglycans (Moulard et al., 2000).

Besides direct interactions of the envelope glycoprotein with cell surface receptors, interactions also occur between cell-derived molecules incorporated onto virions and their ligands. Such interactions enhance the overall efficiency of virus entry. Examples include the integrin ICAM-1 (intercellular adhesion molecule-1), which assembles onto HIV particles (Paquette et al., 1998) and enhances attachment to cells expressing its ligand, LFA-1 (lymphocyte function-associated antigen-1) (Fortin et al., 1999).

Although HIV may attach to cells via a number of distinct interactions, fusion will not occur until sufficient CD4 and coreceptor molecules are recruited to trigger formation of a fusion pore. Thus, direct and early interactions with CD4 are likely to be the most efficient infection process with the fastest kinetics.

Fusion mechanism

HIV particles fuse with cell surface membranes. Envelope/coreceptor engagement triggers gp41 rearrangement and exposure of the fusion domain leading to fusion. The crystal structures of the extracellular gp41 domains have been determined (Chan et al., 1997; Weissenhorn et al., 1997). These gp41 structures represent the triggered fusion-activated form and have a rod-like structure with a bundle of six helices at their core. Similar TM structures have been reported for unrelated enveloped viruses, including influenza virus and Ebola virus, consistent with conserved mechanisms for fusion (Dutch et al., 2000). Three leucine zipper domains (each from one oligomer of the trimer) form a coiled-coil structure that extends up towards the N-terminal fusion domain. Three α-helices proximal (but external) to the virion membrane interact with outer grooves of the coiled coil in an anti-parallel manner to form the six-helix bundle. The conformational events and
Table 1. Cell surface receptors implicated in binding HIV virions

Receptors implicated in binding HIV virions. Receptors other than CD4 or coreceptors that attach HIV virions to cell surfaces.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Affinity ($K_d$)</th>
<th>Expression</th>
<th>Role in attachment and infection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal-C</td>
<td>High (11.6 nM)</td>
<td>Neuronal and glial cells</td>
<td>Confers inefficient infection presumably by aiding attachment</td>
<td>Harouse et al. (1991)</td>
</tr>
<tr>
<td>Sulphatide (sulphate derivative of Gal-C)</td>
<td></td>
<td>Colorectal epithelial cells and primary macrophages</td>
<td>Confers efficient CD4-independent infection by NDK, a TCLA HIV-1 strain</td>
<td>Fantini et al. (1993); Seddiki et al. (1994); Delezay et al. (1997)</td>
</tr>
<tr>
<td>Placental membrane-binding protein</td>
<td>High (1-3-0-6 nM)</td>
<td>Cloned from a placental cDNA library</td>
<td>Binds virus particles to the cell surface and thus enhances infectivity via CD4 and coreceptors. May trap HIV in the periphery and carry to T-cells in lymph nodes</td>
<td>Curtis et al. (1992); Geijtenbeek et al. (2000a)</td>
</tr>
<tr>
<td>DC-SIGN</td>
<td></td>
<td>On dendritic cells</td>
<td>Acts in the same way as DC-SIGN</td>
<td>Pohllmann et al. (2001)</td>
</tr>
<tr>
<td>DC-SIGNR</td>
<td></td>
<td>Endothelial cells, such as liver, sinusoidal and lymph node sinus endothelial cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannose-specific macrophage endocytosis receptor</td>
<td></td>
<td>Macrophages</td>
<td>Binds gp120</td>
<td>Larkin et al. (1989)</td>
</tr>
<tr>
<td>Heparans</td>
<td></td>
<td>Many cell types</td>
<td>Attaches virus particles to cell surfaces via an interaction with the V3 loop thus enhancing infectivity via CD4 and coreceptors. Acts predominantly for CCR5-using viruses</td>
<td>Mondor et al. (1998)</td>
</tr>
<tr>
<td>LFA-1/ICAM-1</td>
<td>LFA-1 is expressed on haematopoietic cells, ICAM-1 is on a wide variety of cell types</td>
<td>ICAM-1 incorporated onto virions enhances attachment and infection of LFA-1+ cells</td>
<td>Fortin et al. (1999); Paquette et al. (1998)</td>
<td></td>
</tr>
</tbody>
</table>

intermediate structures that lead to the formation of the six-helix bundle are not clear. An extended coiled coil may form first, protruding the fusion domain towards the cell membrane. Complexing with the membrane proximal α-helices would then position the two membranes close together (Fig. 3).

Several glycoprotein spikes form a ring and cooperate in the induction of a fusion pore. For influenza virus, three to six HA trimers are required (Danielli et al., 1996). Fusion proceeds from initial curvature of target and virion membranes, following insertion of the fusion peptide, to a short-lived hemifusion stage where only the outer lipid bilayers are fused and, finally, form a flickering pore that stabilizes and expands. Low pH-treated influenza virions usually carry HA1s that appear to be completely disorganized. This disordered state may prevent HA1 from physically hindering the two membranes approaching each other. The fate of gp120, CD4 and coreceptors following gp41 activation is not known. Neither is it clear if gp120 or the receptors play a role in establishing the ring of envelope spikes around the fusion pore or have roles in uncoating events immediately after fusion, as suggested by Chackerian et al. (1997).

The major receptor, CD4

Early evidence that CD4 was the receptor for HIV-1 included the following observations: (1) infection was inhibited by monoclonal antibodies (MAbs) to CD4; (2) expression of CD4 on resistant cells conferred sensitivity to infection; and (3) CD4 could be coprecipitated with the HIV envelope.

CD4 is a ligand for MHC class II molecules interacting with the β2 subunit. It is expressed predominantly on T-helper cells acting as an accessory receptor in the cellular immune response. Its role is to increase the avidity between helper T-cells and MHC class II+ antigen-presenting cells, forming part of a ternary complex with the T-cell receptor (TCR) in antigen recognition. CD4–MHC class II interactions also have roles in cell adhesion, enabling other receptor/ligands to contact.

CD4 is a member of the immunoglobulin superfamily and has four extracellular immunoglobulin-like domains (D1 to membrane proximal D4), a TM region and the cytoplasmic tail that associates with the kinase p56lck. The extracellular domain of CD4 extends 120 Å, the distance required to span the length of the TCR and interact with MHC class II molecules (Janeway,
CD4 site that contacts gp120 forms a charged ridge on the N-terminal domain furthest from the cell membrane. This site is part of the CDR2-like region that corresponds to the second of three complementarity-determining regions (equivalent to the antigen-binding site on antibody molecules). F$^{43}$ and the positive R$^{38}$ residues in this region make multiple contacts with gp120 residues, including negatively charged D$^{388}$, E$^{370}$ and hydrophobic W$^{127}$. The F$^{43}$ side chain penetrates a hole on gp120 (Fig. 4A). About 63% of gp120–CD4 contacts are made by CD4 residues 40–48 (Kwong et al., 1998). The contact gp120 residues are derived from several discontinuous sequences and include conserved amino acids where the backbone of the polypeptide chain, rather than amino acid side chains, contacts CD4. Binding of gp120 to CD4 causes rearrangement of the gp120 core (Myszka et al., 2000) and movement of variable loops resulting in formation of fusion and/or exposure of a site that binds a coreceptor. The crystals determined by Kwong et al. (1998) reveal the structure of gp120 and CD4 complexed together; however, the native gp120 structure prior to CD4 binding remains less clear.

CD4 itself undergoes conformational changes on binding gp120. The crystal structure of gp120–CD4 complexes do not reveal any rearrangements in domain D1D2 compared to uncomplexed CD4 (Kwong et al., 1998). However, CD4 has flexible regions between domains D2 and D3, as well as between domain D4 and the membrane. Such flexibility may be required for CD4 to ‘approach’ gp120 laterally and to orientate the coreceptor-binding site towards the cell surface (Fig. 2). Yachou & Sekaly (1999) showed that gp120 binding resulted in the loss of epitopes on domains D3 and D4 of CD4, consistent with conformational alterations distant from the gp120-binding site. There is evidence that both flexible domains are important for HIV infection. First, MAbs to the D2–D3 hinge block HIV infection but not gp120 binding to CD4 (Healey et al., 1990) and, second, deletions in the D4 membrane-flexible region delay infection and reduce V3 loop exposure, suggesting that conformational changes in gp120 are inefficient without CD4 flexibility (Moir et al., 1996).

**Binding site on CD4 for MHC class II molecules**

The sites on CD4 that interact with MHC class II molecules are complex and encompass a larger surface area compared to the gp120-binding site (reviewed by Ravichandran et al., 1996). Amino acids clustered along one side of CD4 domain D1 in CDR1 and CDR3 as well as domain D2 residues are involved in interaction with MHC class II molecules (Moebius et al., 1993). Evidence indicates that the CDR2 region on the opposite face of CD4 also binds MHC class II (Huang et al., 1997; Moebius et al., 1993) and may be involved in interactions that allow hetero-oligomers to form for augmentation of T-cell activation signals (Huang et al., 1997). Additional evidence suggests a direct association between CD4 and the TCR via the membrane proximal D3 and D4 domains of CD4 (Vignali et al., 1996).
**IL-16**

IL-16 was reported to form homodimers that then interact with the membrane-proximal D4 domain on dimeric CD4 (Liu et al., 1999b). The ability of peptides derived from the D4 domain of CD4 to block IL-16-induced activation is consistent with domain D4 as the IL-16-binding site (Liu et al., 1999b).

**Human herpes virus-7 (HHV-7) exploits CD4 as a receptor**

HHV-7 uses CD4 as a receptor for entry into cells and is inhibited by soluble gp120 as well as by CD4 MAbs (Lusso et al., 1994). However, CD4 transfection did not confer HHV-7 sensitivity for all cell lines (Yasukawa et al., 1997), perhaps indicating that HHV-7 requires a coreceptor for infection. If a coreceptor is required, it is not one of the major HIV coreceptors, CCR5 or CXCR4. Neither is needed for HHV-7 infection (Yasukawa et al., 1999).

**Coreceptors**

Soon after CD4 was shown to be the main receptor for HIV and SIV, evidence started to accumulate indicating that CD4 alone was not sufficient for HIV to fuse with the cells. Maddon et al. (1986) showed that CD4 expressed on mouse cells allowed virus to bind but did not confer virus entry. Data from this study (Maddon et al., 1986) suggested that mouse cells lacked a cofactor or coreceptor needed in addition to CD4 to trigger HIV entry. Of particular importance were the observations that HIV-1 isolates fell into two distinct groups depending on their biological properties. Asjo et al. (1986) described the two groups as slow-low and rapid-high depending on their replication rates in PBMNCs. Related reports described the two virus groups as nonsyncytium-inducing (NSI) or syncytium-inducing (SI) (Tersmette et al., 1988) as well as macrophage-tropic (or M-tropic) and T-cell tropic (or T-cell line tropic, T-tropic) (Gartner et al., 1986). The differences between the two types of isolate were determined by gp120 env sequences and were located predominantly in the V3 loop (Hwang et al., 1991). This evidence was consistent with the two virus groups requiring different cell surface coreceptors for entry.

E. A. Berger and colleagues cloned the first HIV coreceptor, CXCR4 (termed fusin) (Feng et al., 1996). Coexpression of CXCR4 with CD4 on mouse cells conferred fusion by SI or T-tropic (but not NSI/M-tropic) HIV-1 strains. Several groups reported CCR5 as the coreceptor for NSI viruses (Alkhatib et al., 1996; Deng et al., 1996; Dragic et al., 1996). CCR5 and CXCR4 are the major HIV-1 coreceptors and all strains can use one (R5 and X4 viruses) or both (R5X4 viruses) to enter CD4+ cells. R5 viruses are predominantly transmitted and persist throughout infection. Viruses that exploit CXCR4 emerge late in disease and can be isolated from up to 50% of AIDS cases.
Both CCR5 and CXCR4 are members of the 7 TM chemokine receptor family. More than a dozen 7 TM receptors have been shown to act as coreceptors on CD4+ cell lines for particular HIV-1 strains. These coreceptors are also chemokine receptors or are closely related orphan receptors. Currently, there is little evidence to suggest that coreceptors other than CCR5 and CXCR4 are used significantly in vivo.

The pattern of coreceptors used by SIV and HIV-2 is different from HIV-1, as expected (Clapham et al., 1991). SIV uses CCR5 but CXCR4 is rarely used (Meister et al., 2001). SIV strains predominantly exploited in the rhesus macaque (AGM), all use CCR5, as do primary isolates from sooty mangabey (SMM). These variants differ in the way that CCR5 is exploited as a coreceptor (Edinger et al., 1997) and in their capacity to exploit low levels of surface CD4 (Bannert et al., 2000). SIVAGM, SIVSMN and SIVAGM strains often use other coreceptors in addition to CCR5, including GPR15/Bob, CXCR6/Bonzo and GPR1 (reviewed by Clapham & Weiss, 1997). Furthermore, the majority of red-capped mangabeys in Gabon are homozygous for a 24 bp deletion in their CCR5 gene and harbour an SIV strain that uses CCR2b and STRL-33 but not CCR5 (Chen et al., 1998b).

GPR15 and CXCR6/Bonzo are used less frequently by HIV-1, while GPR1 is rarely exploited. Unlike the closely related SIVMAC/SIVSMN group viruses, HIV-2 variants that use CXCR4 do evolve in vivo, probably (as for HIV-1) during the later stages of disease. A minority of HIV-2 strains appear to use CXCR4 exclusively (Guillon et al., 1998; Reeves et al., 1999); however, most primary HIV-2 isolates use a much broader range of coreceptors compared to HIV-1 (Bron et al., 1997; McKnight et al., 1998; Morner et al., 1999). Coreceptors used by HIV-2 in the asymptomatic stage of disease are less clear, since virus loads are often very low and isolates are difficult to culture. R5 HIV-2 strains have been reported (Mormor et al., 1999; Reeves et al., 1999); however, it is not certain whether such strains predominate during the asymptomatic phase and are preferentially transmitted over broadly tropic viruses. Table 2 lists 7 TM receptors identified as coreceptors for HIV and SIV strains in vitro.

### Table 2. Coreceptors that support primate lentivirus infection of CD4+ cell lines in vitro

<table>
<thead>
<tr>
<th>Coreceptor</th>
<th>Ligand</th>
<th>HIV-1</th>
<th>HIV-2</th>
<th>SIV</th>
<th>Reference for coreceptor use</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR1</td>
<td>MIP-1α, MIP-1β, MCP-3, RANTES</td>
<td>-*</td>
<td>+</td>
<td>+</td>
<td>Bron et al. (1997); McKnight et al. (1998)</td>
</tr>
<tr>
<td>CCR2b</td>
<td>MCP-1, MCP-2, MCP-3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Doranz et al. (1996)</td>
</tr>
<tr>
<td>CCR3</td>
<td>Eotaxin, eotaxin-2, MCP-3, MCP-4, RANTES</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Choe et al. (1996); Doranz et al. (1996)</td>
</tr>
<tr>
<td>CCR4</td>
<td>MDC, TARC, RANTES, MIP-1α</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>McKnight et al. (1998)</td>
</tr>
<tr>
<td>CCR5</td>
<td>MIP-1α, MIP-1β, RANTES, MCP-2</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>Alkhatib et al. (1996); Deng et al. (1996); Dragic et al. (1996)</td>
</tr>
<tr>
<td>CCR8</td>
<td>I-309</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Rucker et al. (1997)</td>
</tr>
<tr>
<td>CCR9</td>
<td>TECK</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Choe et al. (1998)</td>
</tr>
<tr>
<td>CXCR2</td>
<td>IL-8, NAP-2, ELR+ CXCs</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>Bron et al. (1997)</td>
</tr>
<tr>
<td>CXCR4</td>
<td>SDF-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Feng et al. (1996)</td>
</tr>
<tr>
<td>CX3CR1/V28</td>
<td>Fractalkine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Alkhatib et al. (1997a); Deng et al. (1997)</td>
</tr>
<tr>
<td>STRL-33/Bonzo/TYMSTR</td>
<td>CXCL16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>GPR1</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Farzan et al. (1997)</td>
</tr>
<tr>
<td>GPR15/Bob</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Farzan et al. (1997)</td>
</tr>
<tr>
<td>API</td>
<td>Apelin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Choe et al. (1998); Edinger et al. (1998)</td>
</tr>
<tr>
<td>RDC1</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Shimizu et al. (2000)</td>
</tr>
</tbody>
</table>

* Rarely or never used as a coreceptor (-); occasional use by a few isolates (+); used by 5–20% of isolates (+ +); frequent use by many isolates or by major subgroup (for example, CXCR4 used by R5X4 and X4 isolates, indicated in bold) (+ + +); major coreceptor used by predominant virus in vivo (for example, CCR5 use by HIV and SIV, indicated in bold) (+ + + + +). NT, Not tested.

The interaction between gp120 and coreceptors

The gp120 sites that interact with coreceptors and determine tropism include the variable V1/V2 and V3 loops as well as a conserved region of β-strands known as the bridging-sheet, which is situated between the V1/V2 and V3 loops (Fig. 4B). Variation in the variable loops may enable HIV to adjust the interaction with coreceptors so that different virus strains...
require subtly distinct sites on coreceptors to trigger fusion. Such variability may aid immune escape but will impact on virus phenotypes determined by coreceptor interactions. Such phenotypes include tropism, sensitivity to inhibition by coreceptor ligands and possibly the use of alternative coreceptors. This section describes the crucial gp120 and coreceptor regions involved in their interaction.

Chemokine receptors form rods in the membrane with a central pore surrounded by the 7TM regions. Bacterial rhodopsin is the only high resolution 7TM structure that has been resolved (Palczewski et al., 2000). Such proteins have four domains exposed on the cell surface: the N terminus and three extracellular loops (E1, E2 and E3). Coreceptors take up different conformations on cell surfaces and on different cell types (Baribaud et al., 2001; Lee et al., 1999), influencing their ability to support HIV infection. Such conformations may result from the formation of dimers, as reported for CCR5 (Lapham et al., 1999) or with heterologous chemokine receptors (Mellado et al., 1999). Associations may also occur with other cell surface molecules, as reported for CCR5 and CD4 (Wu et al., 1996; Xiao et al., 1999). Coreceptor sites involved in HIV entry are centred on the N terminus and E2. Mutagenesis studies showed the N terminus of CCR5 is important for coreceptor activity for HIV-1 R5 viruses (Hill et al., 1998). R5 strains, however, differ in their use of CCR5, as highlighted by the variation in their capacity to infect cells expressing different chimeric human/mouse CCR5 receptors (Picard et al., 1997a). MAbs that bind the N terminus of CCR5 are most efficient at inhibiting gp120 binding, while E2-specific MAbs are potent inhibitors of fusion and infection (Lee et al., 1999; Olson et al., 1999; Wu et al., 1997a). For SIV, both M-tropic and T-tropic strains use CCR5; however, the former require the N terminus of CCR5, while E2 is crucial for T-tropic SIV strains (Edinger et al., 1997). It is unclear if there are CCR5-using HIV-1 strains with the properties of T-tropic SIV strains.

For X4 strains, E2 is critical. Deletion of the N terminus of CXCR4 affects some but not all strains (Picard et al., 1997b), although, when present, participates in binding gp120 (Doranz et al., 1999). Chimeric coreceptors support X4 virus entry providing E2 is present (Lu et al., 1997); however, Brelot et al. (1999) showed that X4 strains vary in their use of CXCR4 E2 with different isoforms isolated from distinct E2 residues for activity.

Electrostatic charge interactions are also likely to enhance gp120–coreceptor interactions. The N terminus of CCR5 (and often other coreceptors) is negatively charged due to three acidic amino acids and four (potentially) sulphated tyrosines, which are important for coreceptor function (Farzan et al., 1998). These negative residues may aid interactions with positive amino acids in and around the bridging sheet on gp120 (Kwong et al., 1998). Moreover, the V3 loops of X4 strains are positively charged, while E2 of CXCR4 contains five negative residues and these oppositely charged faces may interact, as suggested by Platt et al. (2001). Mutagenesis of all five acidic residues does not eliminate HIV infection (Wang et al., 1998). Thus negatively charged residues at the N terminus of CCR5 and in E2 of CXCR4 may enhance their use by electrostatic interactions with R5 and X4 strains, respectively; however, they may not determine the specificity of the interaction.

The coreceptor-binding site on gp120 involves the conserved bridging-sheet that lies between the protruding V1/V2 and V3 loops, as well as some residues in V3 itself (Kwong et al., 1998). Antibodies to both regions block gp120–coreceptor interactions (Trkola et al., 1996; Wu et al., 1996). The V3 loop has long been known as a determinant of tropism and now coreceptor usage. Positive residues in V3 that confer an SI phenotype correlate with the use of CXCR4. The role of the V1/V2 loops in the coreceptor interaction is less clear, since an HIV-1 mutant with deleted V1/V2 loops was infectious (Cao et al., 1997), while recombbinant gp120 deleted for V1/V2 bound coreceptors (Wu et al., 1996). However, when present, V1/V2 loops influence tropism (Koito et al., 1994; Westervelt et al., 1991) and coreceptors used (Cho et al., 1998; Ross & Cullen, 1998). Sites in the V1/V2 loops, the bridging-sheet and the V3 loop may contribute to at least two specific interactions with coreceptors centred on the N terminus and E2. A ‘high affinity’ interaction at both sites may not be needed to trigger infection and may explain why the specificity of the coreceptor interaction can be predominantly mapped to either the N terminus or E2. In summary, diverse virus strains vary in the sites and specific amino acids of coreceptors that they exploit for recognition and triggering fusion. The capacity of HIV to vary the Env and coreceptor residues involved in their interaction will be a major mechanism of immune evasion.

Chemokine binding to chemokine receptors

Chemokines are chemoattractant proteins with roles in immune development, inflammation, immunity, embryogenesis and development. Chemokines are 70–120 residue polypeptides with a common folding pattern that forms an α-helix underlying three anti-parallel β-strands and a less-structured N terminus. The first N-terminal loop carries receptor-binding specificities and, once bound, the N terminus itself is thought to interact with a second receptor site to induce receptor activation and signalling. As for gp120, both the N terminus and the E2 of chemokine receptors are implicated in chemokine binding. The gp120- and chemokine-binding sites overlap but can be separated by mutagenesis. For detailed descriptions of chemokine and chemokine receptor structure and function, see the review by Rojo et al. (1999).

Does HIV signal through CD4 or coreceptors?

Signals induced by HIV interacting with CD4 and/or coreceptors may prepare the intracellular environment for early replication steps during virus entry or modify cell
conditions for virion production when newly made envelopes are trafficking onto the cell surface. Inactivated virions or cross-linked gp120 signal via CD4 and p56lck, inducing nuclear translocation of NF-κB on primary lymphocytes to promote cell cycle progression and commitment for virus production (Briant et al., 1996). Corbeil & Richman (1995) reported that apoptosis of CD4+ T-cells triggered during late stages of replication (Corbeil & Richman, 1995) does not occur in cells expressing CD4 receptors lacking the signalling capacity of their cytoplasmic tail (Corbeil et al., 1996). Soluble forms of both R5 and X4 envelope glycoproteins signal via CCR5 or CXCR4 in vitro (Davis et al., 1997; Hessgasser et al., 1997; Weissman et al., 1997) and focal adhesion kinase was shown to associate with CCR5 following gp120 binding (Cicala et al., 1999). Whether a single virion ligates sufficient receptors to induce a signal during entry remains controversial. However, Arthos et al. (2000) reported that the capacity of viral envelopes to signal via CCR5 on macrophages correlated with the ability of viruses to undergo early post-fusion events.

Signalling per se is not needed for coreceptors to function for virus entry, since the pertussis toxin blocks signal induction but not HIV infection on CD4+ cell lines (Aramori et al., 1997). Truncation of the CCR5 cytoplasmic region or mutation of the DRY motif both block signal transduction but do not effect the capacity of CCR5 to act as a coreceptor (Gosling et al., 1997). Despite the observations of J. Corbeil and colleagues, signalling by newly synthesized envelopes will be minimized by several mechanisms (Vpu- and Nef-induced) that downregulate CD4. Loss of CD4 will prevent efficient Env–coreceptor interactions and signalling during late stages of replication. Finally, shed gp120 may interact with cell surface receptors on uninfected cells and induce signals; however, it is not known whether sufficient concentrations of shed gp120 are present in vivo.

**Cell tropism of HIV in immune and nonimmune tissues**

*In vivo*, HIV replication is restricted to haematopoietic cells that express CD4 and either CCR5 or CXCR4. HIV therefore contrasts with other highly lymphotropic retroviruses, such as human T-lymphotropic virus type I, where virus receptors are expressed on diverse cell types (Weiss et al., 1985). For other retroviral receptors see the review by Sommerfelt (1999). The main cells infected by HIV are T-helper lymphocytes, macrophages and DCs. *In vitro*, R5 viruses infect primary cultures of both lymphocytes and macrophages (Berger et al., 1998), while X4 isolates also infect T-cell lines. The capacity of X4 strains to infect macrophages is controversial. However, we and others have shown that primary X4 isolates infect at least some populations of macrophages (Simmons et al., 1996; Valentin et al., 1994). In the blood of individuals that carry R5 viruses, the CD4+CD45RO+ memory T-cells carry most of the provirus load, although CD45RA+ naive cells are also infected. When CXCR4-using strains emerge, their tropism is broader and new cell populations are targeted. On T-cells, CCR5 expression is mainly restricted to memory cells, while CXCR4 expression is widespread and predominates on naive T-cells (Bleu et al., 1997). Symptomatic, X4 virus-carrying individuals have an increased provirus load in naive T-cells consistent with an expanded T-cell tropism (Blaak et al., 2000; Ostrowski et al., 1999). Early studies suggested that monocytes were infrequently colonized in vivo (Schnittman et al., 1989). However, recent reports indicate that monocytes harbour replication-competent viruses in patients treated with highly active anti-retroviral therapy (HAART) (Lambotte et al., 2000).

Whether DCs are infected has been controversial. Blood DCs form two distinct populations: CD11c+ myeloid and CD11c− plasmacytoid. Both populations express CD4, CCR5 and CXCR4 and support at least some level of HIV replication in vitro (Patterson et al., 2001). Their sensitivity to infection and extent of replication depends on their stage of maturation and phenotype (Bakri et al., 2001; Granelli-Piperno et al., 1998; Patterson et al., 1999). Blood plasmacytoid DCs are more sensitive to both HIV R5 and X4 viruses than myeloid DCs (Patterson et al., 2001). Immature myeloid dendritic cells were reported to selectively support replication by R5 viruses (Granelli-Piperno et al., 1998; Reece et al., 1998; Zaitseva et al., 1997). More mature cells are permissive to R5 and X4 virus entry; however, replication blocks prior to (Granelli-Piperno et al., 1998) and after (Bakri et al., 2001) provirus integration are described. Immature DCs, such as Langerhans’ cells at mucosal membranes, may be the first cells encountered by transmitting HIV. Such maturing cells potentially carry HIV either as DC-SIGN-trapped virus (Geijtenbeek et al., 2000; Masurier et al., 1998) or as infected cells to lymph nodes, where association with T-cells provides a potent medium for the rapid amplification of progeny virus.

Chemokines also influence the types of cells that become infected (Cocchi et al., 1995). Several CD4+CCR5+ T-cell clones from uninfected and nonprogressing HIV-1− individuals were resistant to infection due (at least in part) to endogenously produced β-chemokines (Saha et al., 1998; Vyakarnam et al., 2001). T-cell clones from AIDS patients were substantially more sensitive to infection by R5 viruses, consistent with an increasing colonization of CD4+CCR5+ T-cells as disease progresses. Along mucosal membranes, there is extensive stromal cell-derived factor 1 (SDF-1) expression and down-regulation of CXCR4 on T-lymphocytes (Agace et al., 2000). Langerhans’ cells taken from under the skin express little surface CXCR4, whereas, on culture, high concentrations of CXCR4 held internally in vesicles are rapidly expressed (Zaitseva et al., 1997). These observations may explain the restricted transmission of X4 viruses across mucosal membranes and why DCs in vitro and away from the SDF-1-rich environment of mucosa support at least the early entry stages of X4 virus replication. Another explanation is needed to explain selective transmission of R5 viruses directly into the blood (Wilkinson et al., 1998). Thus, soluble factors such as
chemokines in the tissue milieu or produced endogenously by target cells have a major influence on tropism.

In nonimmune tissues and organs, resident-specialized macrophages carry the virus load; for example, in the liver, HIV antigens are detected in Kupffer cells. The brain is colonized by HIV-1 early in infection and eventually results in dementia or related pathology in up to 30% of AIDS cases. The brain is physically isolated from the blood by the blood–brain barrier, a system of tight, gap junctions between endothelial cells in blood capillaries. HIV is probably carried into the brain by infected monocytes, macrophages or activated T-cells. The main brain cell types infected are perivascular macrophages and microglia (reviewed by Gabuzda & Wang, 2000). Astrocytes do not express CD4 but may be occasionally infected in neonates (Saito et al., 1994). Whether HIV-1 adapts to use brain-expressed coreceptors for replication in brain cells is unclear. Neurotropic and neurovirulent SIV variants have been isolated from infected macaques (Zink et al., 1998). However, it is not known if equivalent variants are involved in HIV-1 brain infection. It is also controversial whether CXCR4-using viruses colonize the brain when they emerge late in disease and the vast majority of virus isolates and envelope sequences from brain tissue indicate that R5 viruses predominate. Recently, Gorry et al. (2001) reported isolation of M-tropic R5X4 and X4 strains from the brain tissue of dementia patients. Whether such CXCR4-using strains are implicated in brain pathogenesis is not known and controversial. However, shed gp120 from X4 viruses has been shown to induce apoptosis of neurons (reviewed by Gabuzda & Wang, 2000). The majority of HIV-1 isolates, including R5 and X4 strains, from blood infect both primary microglial cells and macrophages in vitro (Ghorpade et al., 1998; He et al., 1997; Hibbitts et al., 1999; Shieh et al., 1998). R5 isolates that replicate more efficiently in microglial cultures have been selected in vitro (Shieh et al., 2000). Enhanced replication in cultured microglia was conferred by mutations in V1, an envelope region associated with coreceptor use. Specific amino acids at particular sites in the V3 loop (or motifs) have also been associated with envelopes in the brain (Power et al., 1994). The significance of such motifs is highly controversial but could be associated with the use of alternative brain-encoded coreceptors or with the differential use of CCR5.

Significance of coreceptors for transmission, replication and pathogenesis in vivo

For HIV-1, current data support a model where R5 viruses predominate early in the asymptomatic phase, before strains able to use CXCR4 and often several other coreceptors (R5X4++) emerge (Scarlatti et al., 1997).

Coreceptor and chemokine polymorphisms

Individuals who are homozygous for a 32 bp deletion (Δ32) in the CCR5 gene are greatly protected from infection whether infection is via sex (Dean et al., 1996), blood contact (Wilkinson et al., 1998) or from mother-to-child transmission (Phippott et al., 1999). The 32 bp deletion results in a premature stop codon and a truncated CCR5 protein that fails to reach the cell surface (Benkirane et al., 1997). Homozygotes are therefore effectively CCR5−. The protection conferred by Δ32 CCR5 homozygosity indicates that S1/X4 strains are rarely transmitted, although a small number of HIV+ Δ32 CCR5 homozygotes has been reported. Where tested, these individuals carry viruses that use CXCR4 rather than alternative coreceptors (Michael et al., 1998). Individuals heterozygous for Δ32 CCR5 are not protected from infection (Huang et al., 1996) but survive longer (Dean et al., 1996). These individuals express lower levels of CCR5 (Wu et al., 1997b), partly due to a halved CCR5 gene dosage but also because the Δ32 CCR5 protein interacts with full-length CCR5 in the secretory pathway and retains it there (Benkirane et al., 1997).

Other human CCR5 polymorphisms include a single change (m303) in the CCR5 gene reported in one family (Quillent et al., 1998). m303 causes a premature stop codon that prevents the expression of CCR5. PBMCs from one affected family member who also carried a Δ32 CCR5 gene (m303/Δ32 CCR5) were resistant to infection by HIV-1 R5 strains. Several other CCR5 single nucleotide polymorphisms (SNPs) result in amino acid substitutions that interfere with β-chemokine binding and/or coreceptor activity (Carrington et al., 1999; Howard et al., 1999). The influence of these rare SNPs on HIV in vivo is not known.

Several polymorphic alleles and SNPs in the CCR5 gene promoter region have been identified (Martin et al., 1998b; McDermott et al., 1998). One allele (CCR5 P1, characterized by a pattern of 10 specific bases at particular sites) has been shown to accelerate disease progression in homozygous individuals (Martin et al., 1998b).

A polymorphism in CCR2b that results in a V64I change in a TM domain slows disease progression. CCR2b V64I has no effect on sexual transmission (Smith et al., 1997), although a protective effect on mother-to-child transmission was reported (Mangan et al., 2000). The V64I change does affect the capacity of CCR2b to act as a coreceptor or signal in response to chemokines (Lee et al., 1998). Protection may be due to another CCR5 promoter polymorphism (−1835) linked to V64I in the adjacent CCR2b gene. The −1835 polymorphism unlinked to CCR2b V64I is rare and, to date, studies disagree on whether it protects or accelerates (Martin et al., 1998b; Mummidi et al., 1998). Mellado et al. (1999) reported that CCR2b V64I, but not wild-type receptors, formed heterodimers with CXCR4 when costimulated by monocyte chemotactic protein 1 (MCP-1) or SDF-1. Such heterodimers may reduce CXCR4 available for HIV infection and thus could explain CCR2b V64I protection.

CXCR4 is indispensable to mammals. In mice, both CXCR4 (Ma et al., 1998; Zou et al., 1998) and SDF-1 (Ma et al., 1998) ‘knockouts’ are lethal. So far, only three rare CXCR4
polymorphisms have been reported that are not linked to pathogenesis (Cohen et al., 1998; Martin et al., 1998a). A polymorphism in SDF-1 (the CXCR4 ligand) gene was reported as protective (Winkler et al., 1998). However, other studies showed a faster disease rate (Mummidi et al., 1998; van Rij et al., 1998) or a more rapid decline in CD4 cell numbers (Balotta et al., 1999). This G to A ‘mutation’ is located in the 3’ noncoding region of SDF-1 mRNA and may influence mRNA stability. Two SNPs in the promoter of the RANTES gene (−471 and −96) also slow disease (Gonzalez et al., 2001; McDermott et al., 2000), while one study found an effect on transmission risk (McDermott et al., 2000). G at −96 led to an increase in RANTES expression providing an explanation for protection (Liu et al., 1999a). Two SNPs in the first intron of macrophage inflammatory protein 1α (MIP-1α) were also reported to influence disease progression (Gonzalez et al., 2001). Together, these observations provide evidence that β-chemokines act protectively in vivo. Table 3 summarizes the known polymorphisms in HIV receptors and their ligands that influence the course of HIV infection (reviewed by Carrington et al., 1999).

### Table 3. Human polymorphisms in chemokine and coreceptor receptor genes that influence HIV infection and disease progression

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR5 Δ32/wild-type</td>
<td>Up to 18% in Caucasians</td>
<td>Slows disease progression</td>
</tr>
<tr>
<td>CCR5 Δ32/Δ32</td>
<td>Up to 1% in Caucasians</td>
<td>Protects against infection</td>
</tr>
<tr>
<td>CCR5 m303 leads to premature stop codon and CCR5 truncated in E1</td>
<td>3/209 healthy donors</td>
<td>In combination with a Δ32 CCR5 allele confers T-cells with resistance to R5 viruses</td>
</tr>
<tr>
<td>CCR5 P1 allele, characterized by a pattern of 10 specific bases at different sites, including A at −2459</td>
<td>43–68%</td>
<td>Accelerates disease progression</td>
</tr>
<tr>
<td>CCR5 A/G at −2459</td>
<td>43–68%</td>
<td>Slows disease progression</td>
</tr>
<tr>
<td>CCR2 V32 is linked to a point mutation in the promoter region of CCR5</td>
<td>10–15% in Caucasians and US Africans</td>
<td>Homozygotes have slower disease progression, even slower if Δ32/wild-type CCR5 or V32 I CCR2 also</td>
</tr>
<tr>
<td>SDF-1 in 3’ untranslated region of mRNA. In SDF-1/Δ but not SDF-1Δ mRNA</td>
<td>16–25%</td>
<td>Faster/slower disease progression depending on genotype and population (Gonzalez et al., 2001). Some protection from transmission if −471A present</td>
</tr>
<tr>
<td>RANTES promoter AC, GC and AG at sites −471, −96 (sites equivalent to −403 and −28 as described by Liu et al., 1999)</td>
<td>Variable depending on population</td>
<td>Faster/slower disease progression depending on genotype and population (Gonzalez et al., 2001)</td>
</tr>
<tr>
<td>MIP-1α intron +113, +459</td>
<td>Variable depending on population</td>
<td></td>
</tr>
</tbody>
</table>

#### X4 virus transmission

The protection from HIV-1 infection conferred by the Δ32/Δ32 CCR5 genotype indicates that viruses using CXCR4 or other coreceptors rarely transmit. X4 strains have less opportunity for transmission since R5 strains predominate in most infected individuals. Extensive SDF-1 expression by mucosal epithelia may act as a barrier to X4 viruses (Agace et al., 2000). Harouse et al. (1999) showed that R5 but not X4 SHIV (Chimeric SIV/HIV-1) strains replicated extensively in rhesus macaque gut lymphoid tissue. Thus, X4 viruses that penetrate the SDF-1 barrier at the mucosa may arrive in associated lymphoid tissue containing T-cells and macrophages with CXCR4 downmodulated and inaccessible. Dual-tropic SI strains that use CCR5 and CXCR4 are very sensitive to inhibition by CCR5 chemokines when CXCR4 is absent (Kledal et al., 1997). Such sensitivity may be sufficient for β-chemokines to prevent R5X4 transmitting via a CCR5 route.

Δ32 CCR5 homozygotes may also be protected from blood transfer that bypasses the mucosa (Wilkinson et al., 1998). This observation suggests that there are restrictions to X4 viruses beyond the mucosa and that an incoming virus may need to establish infection at particular site(s) for transmission to be successful. Perhaps a critical tissue or site is permissive for R5 strains but not X4 viruses. Again, observations by Harouse et al. (1999) that gut lymphoid tissue in macaques supports extensive replication by R5 but not X4 SIV/HIV viruses provide a precedent.

#### Suppression of X4 strains?

R5 virus replication and variation would be expected to generate variants that can use CXCR4 in a short period of time. Yet, SI/X4 viruses apparently do not always evolve in vivo, can be isolated from only about 50% of AIDS patients and infrequently from HIV-1 subgroup C-infected individuals
(Tscherning et al., 1998). X4 viruses are not isolated from SIV<sub>MAC</sub>-infected rhesus macaques, even though some SIV strains can use CXC4R in vitro (Owen et al., 2000). It is not known if undetectable levels of CXC4R-using viruses are always present. Regardless, the mechanisms that prevent X4 viruses from predominating in vivo are not understood. Whatever the nature of the restriction, it breaks down and/or is breached during the later stages of disease when CXC4R-using viruses emerge in HIV-1-infected individuals. Valentin et al. (1998) described how IL-4 downregulates CCR5 while upregulating CXC4R and enhancing HIV expression. Thus, IL-4 may select for X4 viruses and against R5 strains, a possibility supported by the observation that HIV<sup>+</sup> individuals carrying a polymorphism in the IL-4 gene promoter that increases expression were more likely to harbour X4 viruses (Nakayama et al., 2000).

Two early studies suggested that SI variants present in the acute phase were later suppressed in favour of NSI viruses at seroconversion (Cornelissen et al., 1995; Lathey et al., 1997) and speculated that SI suppression was due to an immune-mediated mechanism (Lathey et al., 1997). However, primary X4 viruses are resistant to neutralizing antibodies as R5 strains, while a role for T-cell immunity is difficult to envisage, since T-cell epitopes on the envelope glycoprotein are few and unlikely to distinguish between R5 and X4 strains (reviewed by Michael & Moore, 1999). The current consensus strongly favours infrequent transmission of CXC4R-using strains and their emergence only late in disease at the peak of virus diversity (Shankarappa et al., 1999). If X4 strains are frequently present at low levels in infected individuals, new therapies aimed at CCR5 may provide X4 viruses with a selective advantage.

**The role of other coreceptors**

The extent to which HIV-1 exploits coreceptors other than CCR5 or CXC4R in vivo is thought to be minimal (Zhang & Moore, 1999). The growing number of different 7TM receptors that support HIV and SIV infection of cell lines in vitro therefore does not accurately predict coreceptor usage in vivo. High-level expression of alternative coreceptors ‘out of context’ on cell lines seems to deliver them to the cell surface in an active form that can confer virus entry. Factors in vivo that may prevent many of the same alternative coreceptors from functioning (as envisaged for CXC4R) are not known. Recent evidence implicates CXC6/Bonzo for HIV-1 infection of some T-cells (Sharron et al., 2000) and CCR8 for thymocytes (Lee et al., 2000). Furthermore, one or more unidentified coreceptors frequently support HIV-2 and SIV infection of primary T-cells and macrophages in vitro (Chen et al., 1998a; Simmons et al., 2000; Sol et al., 1997; Zhang et al., 2000). Despite these observations, there is no evidence yet to indicate that coreceptors other than CCR5 or CXC4R significantly influence HIV or SIV replication in vivo.

**CD4-independent infection**

Many reports describe CD4-independent infection of various cell types by HIV-1 in vitro (reviewed by Clapham et al., 1996). Such infection is generally inefficient, although HIV-1 and HIV-2 variants selected in vitro are substantially more proficient (Clapham et al., 1996; Hoffman et al., 1999). These variants interact directly with coreceptors. Primary HIV-2 isolates generally infect CD4<sup>+</sup> coreceptor<sup>+</sup> cells more efficiently than HIV-1 (Reeves et al., 1999). CD4-independent infection, however, is ultrasensitive to inhibition by neutralizing antibodies as well as coreceptor ligands (Puffer et al., 2002) and evidence that CD4<sup>+</sup> cells are infected with any frequency in vivo is limited.

**Therapies targeted at HIV entry**

HAART has very effectively reduced virus loads in many HIV<sup>+</sup> individuals, 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 if particular drugs are not tolerated. Many novel strategies that interfere with the HIV entry pathway are being developed.

**CD4**

Intervention of the interaction between CD4 and the HIV envelope is an attractive therapeutic approach, since all HIV and SIV strains bind CD4, while infection without CD4 is probably insignificant in vivo. Therapies based on soluble forms of CD4 were excellent in vitro inhibitors of TCLA HIV-1 strains (Clapham et al., 1989) but failed to influence HIV replication in vivo (Schooley et al., 1990). The sensitivity of TCLA viruses was due to the capacity of soluble CD4 to tear gp120 off virions (Moore et al., 1990). Primary isolates of HIV-1 (R5 or X4 viruses) were substantially more resistant to soluble CD4 (Daar et al., 1990), partly because they had a lower affinity for CD4 but also because gp120 was more stably attached to virions (Moore et al., 1992). New strategies will come from the reported structure of gp120–CD4 complexes (Kwong et al., 1998). For instance, a cavity at the surface of gp120 was revealed that accommodates the phenyl ring of F<sub>43</sub> on CD4. Agents designed to block this cavity may interfere with the interaction between gp120 and CD4 and resulting conformational changes.

**IL-16**

Anti-HIV strategies based on IL-16 have been proposed (Baier & Kurth, 1997). IL-16 blocks HIV-1 infection in vitro by mechanisms that include, for example, inhibition of HIV promoter activity (Zhou et al., 1997), although inhibition of virus entry into macrophages was reported (Truong et al., 1999). In vivo, IL-16 serum levels increase following HIV-1 infection but drop sharply during the late stages of disease (Amiel et al., 1999). T-cell clones derived from long-term
nonprogressors produce elevated levels of IL-16 along with β-chemokines and the unidentified CD8 antiviral factor (Scala et al., 1997). Therapeutic approaches that replenish IL-16 include gene therapy strategies where stem cells are engineered to constitutively produce IL-16 (Zhou et al., 1997) or simply by exogenous administration (Viglianti et al., 1997). IL-16, however, has potent proinflammatory effects and may be toxic in vivo (Viglianti et al., 1997). No clinical trials have been reported yet.

**Sulphated sugars**

Various sulphated sugars block HIV infection in vitro, including heparin (Ito et al., 1987), dextran sulphate (Mitsuya et al., 1988) and curdlan sulphate (Kaneko et al., 1990). Such agents (heparin, for example) block infection by interacting with sites on gp120, including the V3 loop, while others, such as dextran sulphate, also prevent gp120 from binding CD4 (Harrop et al., 1994). These agents are not specific for HIV and also block other retroviruses that use different receptors (McClure et al., 1992). Initial clinical trials with such agents have not reported major influences on virus load or patient health. One study did report reductions in viraemia during and following intraperitoneal administration of dextran-2-sulphate. The mechanism of action, however, is unclear and stimulation of macrophages per se rather than inhibition of HIV entry may be a factor (Shaunak et al., 1998). Regardless, sulphated sugars are neither potent nor specific inhibitors of HIV replication in vivo and it is unlikely that they will be used widely in therapies.

**gp41**

An exciting approach aims to block conformational changes in the envelope that lead to fusion. Peptides derived from the leucine zipper-like domain and the membrane proximal x-helix of gp41 are efficient inhibitors of infection in vitro. Peptides derived from either region are thought to block complexing of the x-helix and leucine zipper and thus inhibit fusion. One peptide, T-20, corresponding to the TM-proximal x-helix is effective in vivo (Kilby et al., 1998) and used as a salvage therapy for patients carrying HIV strains resistant to current inhibitors. It is unlikely that peptides like T-20 will be generally exploited for therapy since they cannot be administered orally and are expensive to prepare. Crystal structures of the complexed gp41 trimers consisting of the leucine zippers and x-helices have identified a hydrophobic cavity between the helices (Chan et al., 1998), providing an opportunity to design small molecules that specifically target and interfere with complex formation and therefore virus fusion (Zhou et al., 2000).

**Coreceptors**

The identification of HIV coreceptors has provided an exciting new therapeutic opportunity. CCR5 is an excellent target for therapy since individuals homozygous for the 32 bp deletion in CCR5 are effectively CCR5+ but healthy. Agents that specifically target CCR5 and block its natural receptor activity should therefore be safe. Moreover, CCR5 antagonists can be potent inhibitors of R5 virus replication in vitro. We reported that a form of RANTES modified at the N terminus (amino-oxy-pentane-RANTES, AOP-RANTES) potently inhibited infection by R5 strains of HIV (Simmons et al., 1997). 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 (Mack et al., 1998). Small organic molecules (800–1000 kDa) that are inexpensive to manufacture and can be taken orally are the best options to target coreceptors. Such small molecules have been successfully used to target 7TM receptors for treating several diseases such as asthma (Kelloway, 1997). The first reported small molecule antagonist of CCR5 (TAK-779) was a potent inhibitor of R5 strains in vitro (Baba et al., 1999). Another CCR5 antagonist (SCH 351125) with improved bioavailability that efficiently blocked R5 virus replication in SCID-hu Thy/Liv mice has been reported (Strizki et al., 2001). AMD3100, a bicyclam derivative, binds CXCR4 and blocks X4 viruses (Donzella et al., 1998). At least some of these agents are already in clinical trials and their success in treating HIV+ patients should be known in the next 1–2 years.

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