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REVIEW ARTICLE

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.

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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 ($< 5{\cdot}5$) 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

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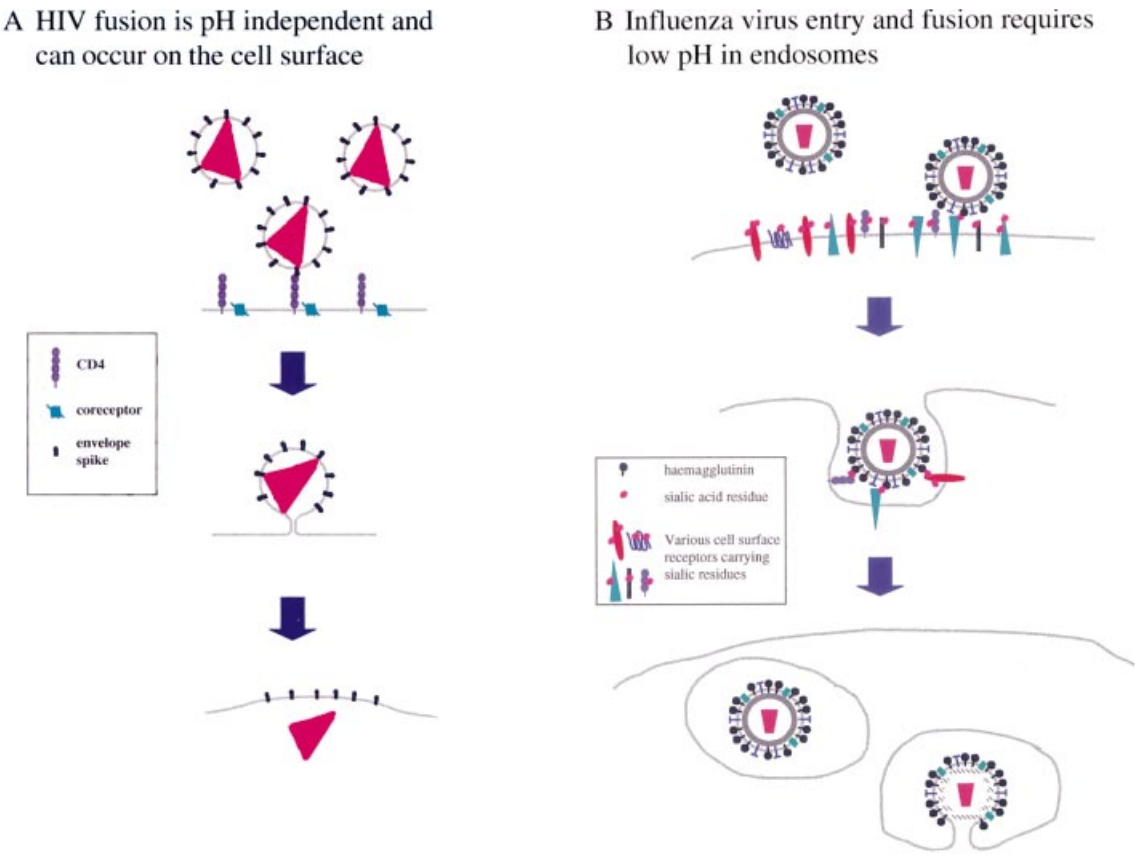

Fig. 1. Attachment and entry into cells by HIV and influenza virus. (A) Sequential interactions between the HIV virion spike glycoprotein and CD4 followed by a coreceptor triggers fusion of the virion and cellular membranes and subsequent virus entry (see Figs 2 and 3 for detailed description). (B) Influenza virions that attach to the cell surface are incorporated into endosomes via coated pits. The low pH in endosomes induces fusion of influenza virus and the endosomal membranes and subsequent virus entry.

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

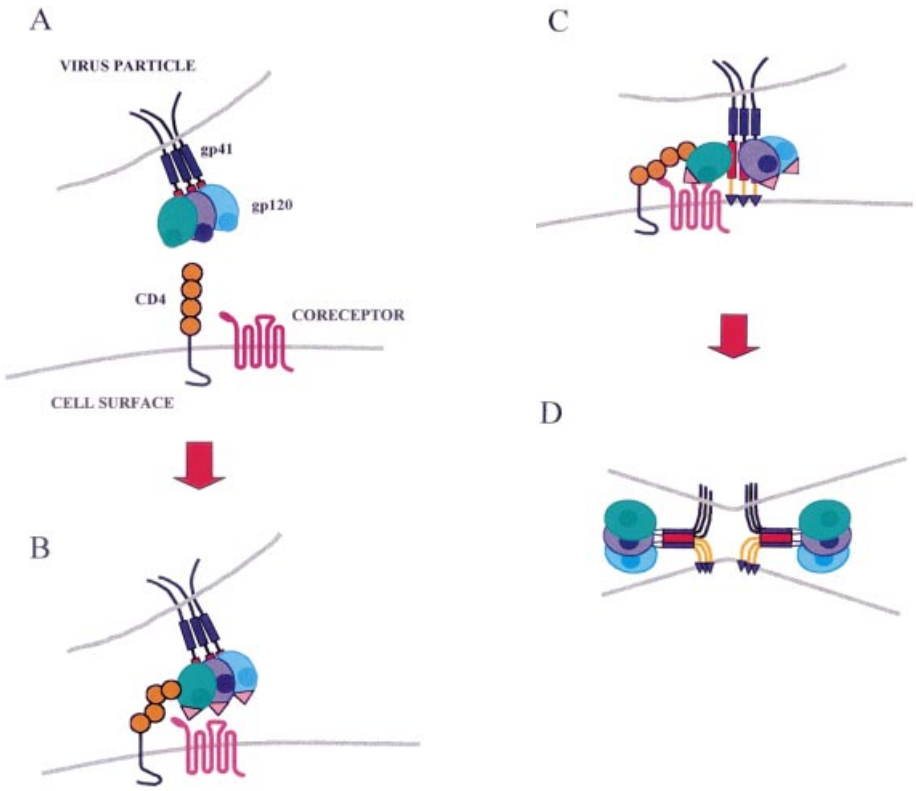

Fig. 2. Events in the attachment and fusion of HIV. (A) HIV virion binds CD4. (B) Conformation changes in the core of gp120 and movement of the variable loops cause exposure or formation of the coreceptor-binding site. Flexible regions in CD4 between domains D2 and D3 as well as domain D4 and the membrane 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.

Receptor	Affinity (K_d)	Expression	Role in attachment and infection	Reference
Gal-C	High (11·6 nM)	Neuronal and glial cells	Confers inefficient infection presumably by aiding attachment	Harouse *et al.* (1991)
Sulphatide (sulphate derivative of Gal-C)		Colorectal epithelial cells and primary macrophages	Confers efficient CD4-independent infection by NDK, a TCLA HIV-1 strain Requires CXCR4 coreceptor	Fantini *et al.* (1993); Seddiki *et al.* (1994); Delezay *et al.* (1997)
Placental membrane-binding protein	High (1·3–0·6 nM)	Cloned from a placental cDNA library	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	Curtis *et al.* (1992); Geijtenbeek *et al.* (2000a)
DC-SIGN		On dendritic cells		
DC-SIGNR		Endothelial cells, such as liver, sinusoidal and lymph node sinus endothelial cells	Acts in the same way as DC-SIGN	Pohlmann *et al.* (2001)
Mannose-specific macrophage endocytosis receptor		Macrophages	Binds gp120	Larkin *et al.* (1989)
Heparans		Many cell types	Attaches virus particles to cell surfaces via an interaction with the V3 loop thus enhancing infectivity via CD4 and coreceptors. Acts predominantly for CXCR4-using viruses	Mondor *et al.* (1998)
LFA-1/ICAM-1		LFA-1 is expressed on haematopoietic cells, ICAM-1 is on a wide variety of cell types	ICAM-1 encorporated onto virions enhances attachment and infection of LFA-1$^+$ cells	Fortin *et al.* (1999); Paquette *et al.* (1998)

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 (Danieli *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 p56^{lck}. 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,

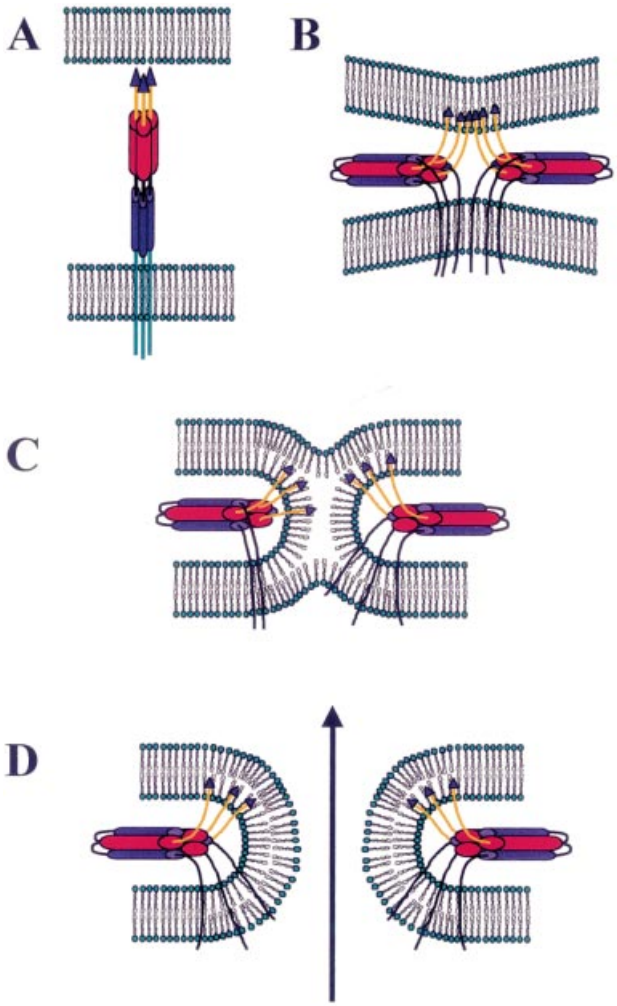

Fig. 3. The role of gp41 in the formation of the fusion pore. (A) Interaction of gp120 with CD4 and a coreceptor exposes the fusion domain (blue triangle). (B) Complexing of the leucine zipper-like region (red) and α-helix (blue) positions the virion and cell membranes close together. Several gp41 molecules are probably needed to form a fusion pore. (C) The outer membranes fuse together at the hemifusion stage. (D) The hemifusion stage is usually short-lived and fusion of the internal membranes follows rapidly. The core inside the virus particle is now exposed to the cell cytoplasm and further uncoating events are needed to allow disassociation from virion membrane and transport of the preintegration complex through the cytoplasm.

1992). CD4 is also expressed on cell types that do not express a TCR, such as monocytes, macrophages and DCs, all of which can be infected by HIV. The role of CD4 on these cells is not well understood; however, CD4 is a receptor for IL-16, a cytokine with chemoattractant activity for $CD4^+$ lymphocytes, monocytes and eosinophils (Cruikshank *et al.*, 1998).

Binding site on CD4 for gp120

The interaction between CD4 and gp120 is conserved among all primate lentiviruses. The site on CD4 that interacts with gp120 has been mapped by mutagenesis and the structure of a gp120–CD4 complex reported (Kwong *et al.*, 1998). The 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^{59} residues in this region make multiple contacts with gp120 residues, including negatively charged D^{368}, E^{370} and hydrophobic W^{427}. 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 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).

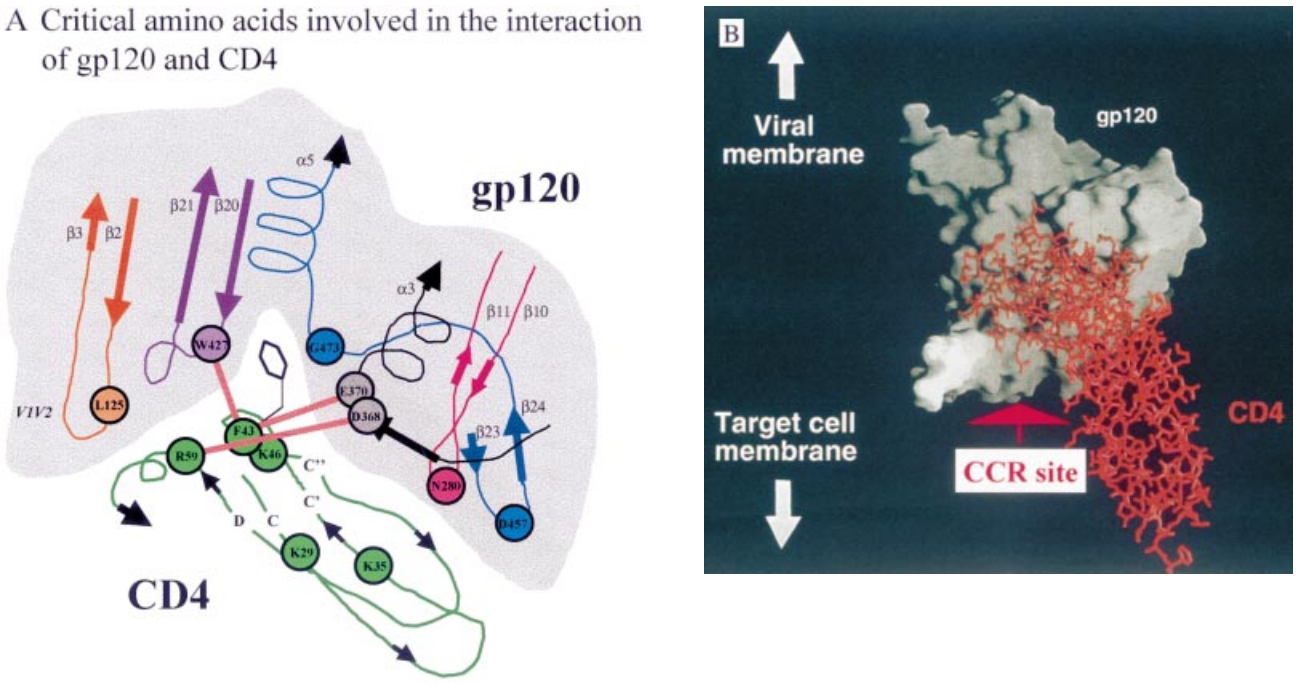

Fig. 4. Receptor-binding sites on gp120. (A) Critical amino acids involved in the interaction of gp120 and CD4. Amino acids from five different domains of gp120 make up the conformational binding site that interacts with CD4. Red bars mark critical contacts between F^{43} on CD4 and W^{427}, E^{370} on gp120 as well as between R^{59} on CD4 and D^{368} on gp120. (B) Structure of gp120 showing bridging sheet situated between V1/V2 and V3 and 'below' the CD4-binding site. The coreceptor binding site is thought to consist mainly of sites on the bridging sheet and V3 loop.

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 PBMCs. 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.

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

Coreceptor	Ligand	HIV-1	HIV-2	SIV	Reference for coreceptor use
CCR1	MIP-1α, MPIF-1, MCP-3, RANTES	–*	+	+	Bron *et al.* (1997); McKnight *et al.* (1998)
CCR2b	MCP-1, MCP-2, MCP-3	+	++	+	Doranz *et al.* (1996)
CCR3	Eotaxin, eotaxin-2, MCP-3, MCP-4, RANTES	++	++	+	Choe *et al.* (1996); Doranz *et al.* (1996)
CCR4	MDC, TARC, RANTES, MIP-1α	–	+	–	McKnight *et al.* (1998)
CCR5	**MIP-1α, MIP-1β, RANTES, MCP-2**	++++	++++	++++	**Alkhatib *et al.* (1996); Deng *et al.* (1996); Dragic *et al.* (1996)**
CCR8	I-309	++	++	++	Rucker *et al.* (1997)
CCR9	TECK	+	–	–	Choe *et al.* (1998)
CXCR2	IL-8, NAP-2, ELR^+ CXCs	–	+	NT	Bron *et al.* (1997)
CXCR4	**SDF-1**	+++	+++	+	**Feng *et al.* (1996)**
CX3CR1/V28	Fractalkine	+	+	+	Reeves *et al.* (1997)
STRL-33/Bonzo/TYMSTR	CXCL16	+	+++	+++	Alkhatib *et al.* (1997a); Deng *et al.* (1997)
GPR1	?	+	++	+++	Farzan *et al.* (1997)
GPR15/Bob	?	+	++	+++	Farzan *et al.* (1997)
APJ	Apelin	+	+	+	Choe *et al.* (1998); Edinger *et al.* (1998)
Chem R23	?	+	–	+	Samson *et al.* (1998)
RDC1	?	+	+	+	Shimizu *et al.* (2000)

* 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.

Both CCR5 and CXCR4 are members of the 7TM chemokine receptor family. More than a dozen other 7TM 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 (MAC) or cynomolgus animal models, such as African green monkeys (AGM), all use CCR5, as do primary isolates from sooty mangabey monkeys (SMM) (Chen *et al.*, 1998a). Several SIV strains have been shown to use CXCR4 on cell lines *in vitro*. In most cases, infection was insubstantial and its significance unclear (Owen *et al.*, 2000; Schols & De Clercq, 1998). A switch from CCR5- to CXCR4-use associated with disease progression has not been reported in SIV animal models. T-tropic and M-tropic SIV_{MAC} strains, however, are apparent. 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). SIV_{MAC}, SIV_{SMM} and SIV_{AGM} 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 SIV_{MAC}/SIV_{SMM} 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 (Morner *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 7TM receptors identified as coreceptors for HIV and SIV strains *in vitro*.

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_{MAC}, 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 isolates dependent on 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 recombinant 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 $p56^{lck}$, 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; Hesselgesser *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-lymophotropic 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 (Bleul *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 antiretroviral 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 $HIV\text{-}1^+$ 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 Küppfer 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 (Philpott *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 SI/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 $V^{64}I$ change in a TM domain slows disease progression. CCR2b $V^{64}I$ has no effect on sexual transmission (Smith *et al.*, 1997), although a protective effect on mother-to-child transmission was reported (Mangano *et al.*, 2000). The $V^{64}I$ 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 $V^{64}I$ in the adjacent CCR2b gene. The −1835 polymorphism unlinked to CCR2b $V^{64}I$ 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 $V^{64}I$, 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 $V^{64}I$ 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

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

Genotype	Frequency	Effect
CCR5 Δ32/wild-type	Up to 18% in Caucasians	Slows disease progression
CCR5 Δ32/Δ32	Up to 1% in Caucasians	Protects against infection
CCR5 m303 leads to premature stop codon and CCR5 truncated in E1	3/209 healthy donors	In combination with a Δ32 CCR5 allele confers T-cells with resistance to R5 viruses
CCR5 P1 allele, characterized by a pattern of 10 specific bases at different sites, including A at −2459	43–68%	Accelerates disease progression
CCR5 A/G at −2459	43–68%	Slows disease progression
CCR2 $V^{64}I$ is linked to a point mutation in the promoter region of CCR5	10–15% in Caucasians and US Africans	Slows disease progression
SDF-1 in 3′ untranslated region of mRNA. In SDF-1β but not SDF-1α mRNA	16–25%	Homozygotes have slower disease progression, even slower if Δ32/wild-type CCR5 or $V^{64}I$ CCR2 also
RANTES promoter AC, GC and AG at sites −471, −96 (sites equivalent to −403 and −28 as described by Liu *et al.*, 1999)	Variable depending on population	Faster/slower disease progression depending on genotype and population (Gonzalez *et al.*, 2001). Some protection from transmission if −471A present
MIP-1α intron +113, +459	Variable depending on population	Faster/slower disease progression depending on genotype and population (Gonzalez *et al.*, 2001)

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).

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_{MAC}-infected rhesus macaques, even though some SIV strains can use CXCR4 *in vitro* (Owen *et al.*, 2000). It is not known if undetectable levels of CXCR4-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 CXCR4-using viruses emerge in HIV-1-infected individuals. Valentin *et al.* (1998) described how IL-4 downregulates CCR5 while upregulating CXCR4 and enhancing HIV expression. Thus, IL-4 may select for X4 viruses and against R5 strains, a possibility supported by the observation that HIV^+ 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 as 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 CXCR4-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 CXCR4 *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 CXCR4) are not known. Recent evidence implicates CXCR6/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 CXCR4 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 $CD4^-$ $coreceptor^+$ 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 $CD4^-$ 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^+ 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^{43} 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 α-helix of gp41 are efficient inhibitors of infection *in vitro*. Peptides derived from either region are thought to block complexing of the α-helix and leucine zipper and thus inhibit fusion. One peptide, T-20, corresponding to the TM-proximal α-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 α-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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