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REVIEW ARTICLE

Human immunodeficiency virus type 2

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Introduction

The AIDS pandemic continues to spread unchecked in many parts of the world, with greater than 34 million individuals currently infected with human immunodeficiency virus (HIV). While most infections are due to HIV type 1 (HIV-1) strains, HIV-2 represents a significant minority of all HIV infections in some countries, such as Guinea-Bissau and Portugal. While similar in many ways, there are important differences between HIV-1 and HIV-2 that provide insights into virus evolution, tropism and pathogenesis. Major differences include reduced pathogenicity of HIV-2 relative to HIV-1, enhanced immune control of HIV-2 infection and often some degree of CD4-independence. This review discusses the origin of HIV-2 and its relationship to simian immunodeficiency virus and HIV-1, its epidemiology, its pathogenic potential and how its Env protein interacts with cell surface receptors to mediate virus infection.

Discovery of human immunodeficiency virus type 2 (HIV-2)

AIDS, resulting from HIV-1 infection, was first recognized in 1981 when a common pattern of symptoms was observed among a small number of homosexual men in the USA (Brennan & Durack, 1981; Gottlieb *et al*., 1981). AIDS cases were soon reported in other groups, including intravenous drug users and haemophiliacs (CDC, 1982; Davis *et al*., 1983; Masur *et al*., 1981). Soon after the identification of AIDS in humans, outbreaks of wasting and severe infections were identified in captive colonies of Asian rhesus macaques in USA primate centres and these symptoms became known as simian AIDS (Henrickson *et al*., 1983; Letvin *et al*., 1983). The sera from these animals showed cross-reactivity to HIV-1 antigens by Western blot (Kanki *et al*., 1985), which led to the identification of a related lentivirus, termed simian immunodeficiency virus (SIV) (Daniel *et al*., 1985). Surprisingly, sera from Senegalese sex workers were found to cross-react preferentially with SIV antigens compared to HIV-1 by Western blot, indicating exposure to an SIV-like virus (Barin *et al*., 1985). Subsequently, a virus more closely related to SIV than HIV-1 was isolated from West African AIDS patients from Guinea-Bissau and Cape Verde (Clavel *et al*., 1986). This virus, referred to as lymphadenopathy-associated virus type 2, is now known as HIV-2.

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Origin of HIV-2 and relationship to HIV-1

HIV-2, along with HIV-1 and SIV, comprise the subgenus ‘primate lentiviruses’. The genomic organization of these viruses is similar, although HIV-1 and SIV of chimpanzees ($\mathrm{SIV_{cpz}}$) encode a *vpu* gene, while HIV-2 and SIV of sooty mangabeys ($\mathrm{SIV_{sm}}$) have a *vpx* gene. The function of Vpx is not clear, although it may play a role in nuclear import (Fletcher *et al*., 1996) and the functions normally associated with HIV-1 Vpr may be provided separately in HIV-2 by Vpr (cell cycle arrest in G_2) and Vpx (nuclear import) in HIV-2. Vpx (but not Vpr) is important for efficient replication of HIV-2 in PBMCs but not T cell lines (Park & Sodroski, 1995). Additionally, $\mathrm{Vpx^-}$ or $\mathrm{Vpr^-}$ SIVs can cause AIDS, while double mutant $\mathrm{Vpx^-/Vpr^-}$ SIVs are severely attenuated (Gibbs *et al*., 1995).

Despite similar genomic organizations, there is a high degree of genetic diversity between the primate lentiviruses, especially in their *env* genes. Genetic variability between HIV-2 strains is comparable to that within HIV-1 groups, with up to 25% divergence in Gag, Pol and Env (Gao *et al*., 1994; Schulz *et al*., 1990; Zagury *et al*., 1988).

HIV is thought to have originated from zoonotic transmissions from SIV-infected non-human primates (Gao *et al*., 1992, 1999; Hirsch *et al*., 1989). SIVs from chimpanzees cluster phylogenetically with HIV-1 (Fig. 1) (Gao *et al*., 1999); hence, the HIV-1 epidemic is likely to have originated from $\mathrm{SIV_{cpz}}$. In contrast, all criteria identifying HIV-2 as a zoonosis from the sooty mangabey (*Cercocebus atys*) are met: i.e. similarity in genomic organization; phylogenetic relatedness (Fig. 1); prevalence in the natural host; geographical overlap; and plausible route of transmission (Sharp *et al*., 1995). From phylogenetic analysis of divergent HIV-2 strains, it appears that there have been seven independent transmissions from sooty mangabeys to humans, resulting in HIV-2 subtypes A–G (Chen *et al*., 1997a; Gao *et al*., 1994; Yamaguchi *et al*., 2000). Only one member each of subtypes C, E, F and G, and two members of subtype D, have been identified (reviewed by Schim van der Loeff & Aaby, 1999) and it is thought that these rare subtypes may be primary zoonotic infections.

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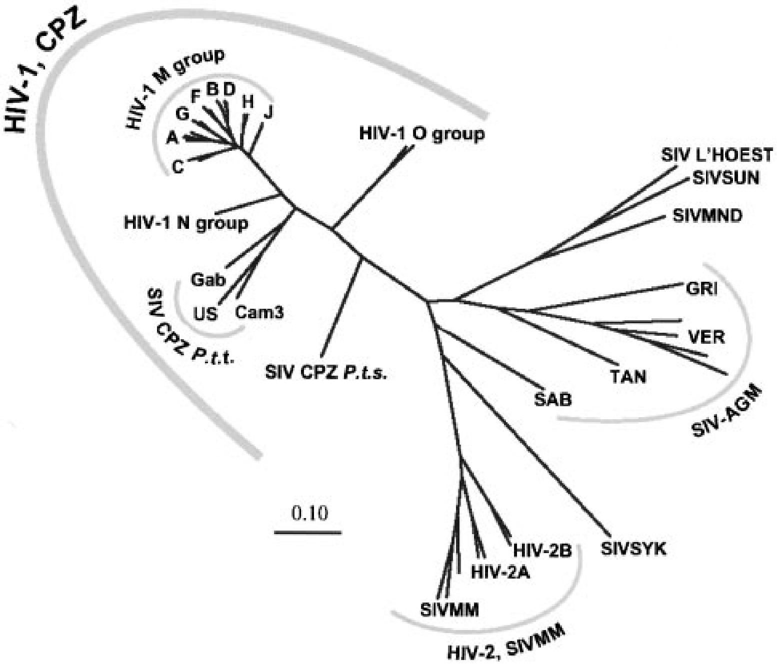

Fig. 1. Phylogenetic relationship of primate lentiviruses. Alignment of *pol* gene sequences of HIV-1, HIV-2 and SIV strain SIV_{mm}; SIV of sooty mangabeys or macaques experimentally infected with SIV_{mm}. Reproduced from *Human Retroviruses and AIDS* (Los Alamos, New Mexico; Theoretical Biology and Biophysics Group, Los Alamos National Laboratory) (Kuiken *et al.*, 1999), with permission.

Epidemiology, transmission and treatment

Epidemiology

The epicentre of HIV-1 infection is East Africa. In contrast, HIV-2 is restricted primarily to West Africa, although the prevalence of HIV-2 is a growing concern in certain parts of Europe and in the southwestern region of India. In Guinea-Bissau, a former Portuguese colony, there is an HIV-2 prevalence of up to 8–10% (Poulsen *et al.*, 1989; Wilkins *et al.*, 1993). A lower prevalence of 1–2% is found in surrounding countries, including The Gambia, Senegal and Guinea, although the prevalence among commercial sex workers in The Gambia is as high as 28% (Ghys *et al.*, 1997; Langley *et al.*, 1996). In addition to West Africa, countries with past socio-economical links with Portugal, including southwest India (Babu *et al.*, 1993; Kulkarni *et al.*, 1992; Rubsamen-Waigmann *et al.*, 1991), Angola, Mozambique and Brazil, have significant numbers of HIV-2 infections (reviewed by Schim van der Loeff & Aaby, 1999). Portugal has the highest prevalence of HIV-2 infection in Europe, accounting for around 10–13% of total HIV infections and 4·5% of AIDS cases (Soriano *et al.*, 2000; UNAIDS/WHO, 1998).

Subtype A accounts for the majority of HIV-2 infections and is the predominant genotype in Guinea-Bissau and Europe (Norrgren *et al.*, 1997; Schim van der Loeff & Aaby, 1999). The prototype HIV-2 strain, ROD, is a subtype A virus that was isolated from a Cape Verdian (Clavel *et al.*, 1986). Subtype B viruses seem to have originated from the eastern parts of West Africa (Ghana and the Ivory Coast) and have occasionally been isolated in Europe (reviewed by Schim van der Loeff & Aaby, 1999). Sierra Leone has a low prevalence of HIV-2, around 0·02%, but the highest diversity of HIV-2 subtypes (A, B, E and F), probably resulting from zoonoses from local sooty mangabeys infected with diverse strains of SIV_{sm} (Chen *et al.*, 1996, 1997a; Gao *et al.*, 1994).

Instances of dual infection ($HIV\text{-}1^{+}/HIV\text{-}2^{+}$) are occurring more frequently in HIV-2 endemic regions, such as Guinea-Bissau, due to the rising prevalence of HIV-1 infection in these areas (Andersson *et al.*, 1999; Ishikawa *et al.*, 1998), raising the possibility that recombination events between HIV-1 and HIV-2 will occur. The epidemiology of HIV-2 is detailed further in a review by Schim van der Loeff & Aaby (1999).

Transmission

Due to differences in transmission rates and virulence, HIV-1 is pandemic, with rising prevalence rates in developing countries, while HIV-2 is more endemic, with stable prevalence rates in most countries (Remy, 1998). The transmission of HIV-2 compared to HIV-1 is detailed elsewhere (Schim van der Loeff & Aaby, 1999). Briefly, HIV-2 appears to be transmitted by the same routes as HIV-1; however, the frequency of transmission is reduced, probably due to a very low virus load in many asymptomatic individuals (Adjorlolo-Johnson *et al.*, 1994; Berry *et al.*, 1998; Cavaco-Silva *et al.*, 1998; Kanki *et al.*, 1994; O'Donovan *et al.*, 2000). For instance, sexual and vertical transmissions of HIV-2 are around 5- to 9-fold and 10- to 20-fold reduced relative to HIV-1, respectively.

Treatment of infection

HIV-2-infected individuals in Europe have been treated with anti-retroviral agents (Smith *et al.*, 2001; Soriano *et al.*, 2000; van der Ende, 2000; van der Ende *et al.*, 2000) but there are no reports of large-scale clinical trials involving HIV-2-infected cohorts. As for HIV-1, the use of single anti-retroviral agents, or suboptimal combination therapy, can lead to the development of drug-resistance mutations *in vivo* (Rodes *et al.*, 2000; Smith *et al.*, 2001; van der Ende *et al.*, 2000). Additionally, many HIV-2 strains naturally have amino acids that confer drug resistance and may thus decrease the therapeutic potential of some anti-retroviral agents (Isaka *et al.*, 2001; van der Ende, 2000). However, others are active against HIV-2 (Clark *et al.*, 1998; Witvrouw *et al.*, 1999) and, with the correct drug combination, the reduced virus load and virulence of HIV-2 may make highly active anti-retroviral therapy extremely effective (Smith *et al.*, 2001; Whittle *et al.*, 1998). The distribution of anti-retroviral drugs within developing countries and the development of an inexpensive vaccine remain priorities.

Pathogenesis and immune response

Naturally infected sooty mangabeys, African green monkeys and chimpanzees do not develop SIV-related disease, even though high virus loads can sometimes be detected in their plasma (Desrosiers, 1990; Rey-Cuille *et al.*, 1998). Disease is only associated with cross-species transmission of the viruses; i.e. from sooty mangabeys into humans (HIV-2) and rhesus macaques (SIV_{mac}) or from chimpanzees into humans (HIV-1). The clinical features of HIV-2 infection are similar to those of HIV-1 infection (Brun-Vezinet *et al.*, 1987); however, HIV-2 is generally less pathogenic. This may be due to differences in virulence, the lower plasma virus load that is usually associated with HIV-2 infection or better immune control of HIV-2 replication compared to HIV-1 (Andersson *et al.*, 2000; Berry *et al.*, 1998; Simon *et al.*, 1993; Soriano *et al.*, 2000; Whittle *et al.*, 1998). HIV-2-infected individuals usually have a long clinically latent period of 10 years or more, resulting in a mortality rate estimated to be two-thirds lower than that for HIV-1 (Marlink *et al.*, 1994; Pepin *et al.*, 1991; Whittle *et al.*, 1994). Indeed, many HIV-2-infected individuals appear not to progress to AIDS at all (Poulsen *et al.*, 1997). When infection does progress, however, virus load and rate of progression are fairly comparable between HIV-1 and HIV-2 (Berry *et al.*, 1998; Simon *et al.*, 1993; Whittle *et al.*, 1992). Interestingly, the burden of proviral DNA is similar in HIV-1 and HIV-2 infections, hence the lower virus load typically observed in HIV-2-infected individuals may be accounted for by differences in virus production (Popper *et al.*, 2000).

It has been reported that HIV-2 infection can prevent/protect ('immunize') against subsequent HIV-1 infection both *in vitro* (Arya & Gallo, 1996; Browning *et al.*, 1999; Dern *et al.*, 2001; Kokkotou *et al.*, 2000; Rappaport *et al.*, 1995) and *in vivo* (Greenberg *et al.*, 1996; Travers *et al.*, 1995, 1998); however, these *in vivo* findings have proved controversial (Aaby *et al.*, 1997; Ariyoshi *et al.*, 1997; Norrgren *et al.*, 1999; Wiktor *et al.*, 1999). Sera from HIV-2-infected individuals are often better at neutralizing autologous as well as heterologous virus in comparison to HIV-1^+ sera and some HIV-2 antisera can cross-neutralize HIV-1 strains (Bjorling *et al.*, 1993; Fenyo & Putkonen, 1996; Robert-Guroff *et al.*, 1992; Weiss *et al.*, 1988). Additionally, HIV-2-infected individuals often have a strong cytotoxic T lymphocyte (CTL) response to HIV-2, which can frequently cross-react with HIV-1 strains (Bertoletti *et al.*, 1998; Gotch *et al.*, 1993; Rowland-Jones *et al.*, 1995). CTL responses to HIV-2 infection are reviewed further by Whittle *et al.* (1998).

The overproduction of β chemokines by PBMCs from HIV-2-infected donors can prevent infection of R5 tropic (see below) HIV-1 strains *in vitro* (Kokkotou *et al.*, 2000). Furthermore, HIV-2 (but not HIV-1) Env protein can interact with CD8 on T cells that are non-permissive to infection, triggering the production of β chemokines (Akimoto *et al.*, 1998; Ichiyama *et al.*, 1999). Higher proportions of $CD8^+$ T cells from HIV-2-infected individuals retain the ability to simultaneously produce the cytokines IL-2 and IFN-γ. Likewise, more $CD4^+$ T cells are capable of producing IL-2 than those from HIV-1-infected individuals with equivalent $CD4^+$ T cell counts (Sousa *et al.*, 2001). These factors may also account in part for the better immune control of HIV-2 infection compared to that of HIV-1 infection. It is also interesting to note that deletions within the *nef* gene of HIV-2 are quite common (Switzer *et al.*, 1998), while *nef* deletions are found infrequently in HIV-1, where they significantly reduce replication *in vitro* and *in vivo* (Piguet & Trono, 1999). Nef downregulates surface expression of MHC class I molecules (Kerkau *et al.*, 1997; Le Gall *et al.*, 1998), thus a functional Nef protein may aid evasion of host immune responses.

The clinical manifestations of HIV-2 AIDS are similar to those for HIV-1 and only minor differences in pathology resulting from HIV-2, compared to HIV-1, infection have been observed. For example, in the Ivory Coast, encephalitis was shown at autopsy to be almost restricted to individuals with HIV-2-related causes of death (18%; $n = 40$), compared to HIV-1 ($< 1\%$; $n = 170$) (Lucas *et al.*, 1993). Whether this is because people infected with HIV-2 generally survive longer than those infected with HIV-1 or if HIV-2 is more neurotropic/neuropathogenic than HIV-1 is unknown. Additionally, AIDS-associated Kaposi's sarcoma occurs in around 10% of HIV-1-infected individuals (Safai, 1997), although it is less frequent in HIV-1-infected Gambians and approximately 12-fold less frequent still in HIV-2-infected Gambians (Ariyoshi *et al.*, 1998).

Envelope structure and function

The *env* genes of HIV-1 and HIV-2 encode the precursor 160 and 140 kDa glycoproteins (gp160/gp140, respectively). These precursors are cleaved by a host protease into the HIV-1 120 kDa and HIV-2 125 kDa surface (SU) glycoproteins (gp120/gp125) and the 41 and 36 kDa transmembrane (TM) glycoproteins (gp41/gp36) for HIV-1 and HIV-2, respectively (Freed *et al.*, 1989; McCune *et al.*, 1988). SU and TM form glycoprotein 'spikes' in the virus membrane that consist of trimers of non-covalently linked SU and TM proteins (Center *et al.*, 2001; Chan *et al.*, 1997; Lu *et al.*, 1995; Weissenhorn *et al.*, 1997). As for HIV-1, the SU protein of HIV-2 contains five variable (V1–V5) and five conserved domains (C1–C5), numerous *N*-linked glycosylation sites and conserved disulphide bonds (Modrow *et al.*, 1987; Starcich *et al.*, 1986; Willey *et al.*, 1986). In addition, residues in HIV-1 gp120 implicated in CD4 or coreceptor binding are highly conserved in SIV and HIV-2 (Kwong *et al.*, 1998; Rizzuto *et al.*, 1998), indicating that these proteins likely share similar structures. HIV and SIV TM proteins consist of an N-terminal extracellular domain, containing a fusion peptide and two α-helical regions, a membrane-spanning domain and a C-terminal intracellular domain with an endocytosis/basolateral targeting signal and two amphipathic regions. Structural studies indicate that the HIV-1 and SIV TM proteins are highly similar (Blacklow *et al.*, 1995; Chan *et al.*, 1997; Lu *et al.*, 1995; Weissenhorn *et al.*, 1997; Yang *et al.*, 1999), making it likely that the HIV-2 TM subunit closely resembles these proteins.

Interaction with CD4

The Env proteins of HIV are primarily involved in binding and entry. SU proteins interact with cellular receptors to attach virus particles to the cell surface and to induce conformational changes in both the SU and TM proteins, which enable triggering of the fusion process. As for HIV-1, CD4 is the primary receptor for all HIV-2 strains. However, a number of HIV-2 isolates have been described that can infect cells independently of CD4 (see below) (Reeves *et al.*, 1999). For most HIV-1 strains, the Env–CD4 interaction is of high affinity, with dissociation constants occurring in the nM range; for example, around 3–4 nM for the HIV-1 IIIB strain (Lasky *et al.*, 1987; Moore, 1990). The interaction of HIV-2 and SIV Env proteins with CD4 may be of somewhat lower affinity, although relatively few virus strains have been examined carefully (Ivey-Hoyle *et al.*, 1991; Layne *et al.*, 1990; Moore, 1990). For example, the dissociation constant of HIV-$2_{\mathrm{ROD/A}}$ SU protein to CD4 is approximately 45 nM, while that for the $\mathrm{SIV}_{\mathrm{mac}}$ SU protein is around 350 nM (Ivey-Hoyle *et al.*, 1991; Moore, 1990).

Interaction with coreceptors

CD4 binding induces conformational changes in the SU subunit of Env that enable it to bind a coreceptor. In the case of HIV-1, CD4 binding induces the formation and/or exposure of a highly conserved domain in the bridging sheet region of the SU protein that has been shown to be important for coreceptor binding (Fig. 2) (Rizzuto *et al.*, 1998). Many residues in this region are conserved between HIV-1, HIV-2 and SIV strains. Thus, it is likely that CD4 binding induces similar conformational changes in HIV-2 Env. As for HIV-1, all HIV-2 strains use either CCR5 and/or CXCR4 as major coreceptors for entry into $\mathrm{CD4}^+$ cells (Bron *et al.*, 1997; Deng *et al.*, 1997; Hill *et al.*, 1997; Pleskoff *et al.*, 1997a, b; Rucker *et al.*, 1997; Sol *et al.*, 1997). Amino acids in the V3 loop of Env can determine X4 or R5 tropism for both HIV-1 and HIV-2 strains (Choe *et al.*, 1996; Cocchi *et al.*, 1996; Isaka *et al.*, 1999; Speck *et al.*, 1997). Many HIV-2 strains can, however, use a wider range of coreceptors compared to HIV-1 and may use these as efficiently as CCR5 or CXCR4 (Guillon *et al.*, 1998; McKnight *et al.*, 1998; Owen *et al.*, 1998; Reeves *et al.*, 1999; Unutmaz *et al.*, 1998). Additionally, some HIV-2 strains can utilize coreceptors that are not, or are rarely, used by HIV-1 strains, including CCR1 (Guillon *et al.*, 1998; McKnight *et al.*, 1998; Owen *et al.*, 1998), CCR4 (McKnight *et al.*, 1998; Owen *et al.*, 1998) and GPR1 (Liu *et al.*, 2000; Shimizu *et al.*, 1999). Other coreceptors used by HIV-2 strains include CCR2b (Guillon *et al.*, 1998; McKnight *et al.*, 1998; Owen *et al.*, 1998), CCR3 (Bron *et al.*, 1997; Reeves *et al.*, 1997; Sol *et al.*, 1997), CCR8 (Rucker *et al.*, 1997; Simmons *et al.*, 2000), CXCR5 (Kanbe *et al.*, 1999), $\mathrm{CX_3CR1}$ (Reeves *et al.*, 1997; Rucker *et al.*, 1997), GPR15 (Deng *et al.*, 1997; Owen *et al.*, 1998), STRL33 (Deng *et al.*, 1997; Owen *et al.*, 1998), RDC1 (Shimizu *et al.*, 2000), APJ (Liu *et al.*, 2000) and US28 (Pleskoff *et al.*, 1997b). Thus, HIV-2 strains are, in general, more promiscuous than HIV-1 strains in their use of coreceptors. However, it is not clear if use of receptors other than CCR5 or CXCR4 is relevant *in vivo*. Some alternative coreceptors are not expressed on $\mathrm{CD4}^+$ cells or are expressed at levels below that needed to support virus infection (Sharron *et al.*, 2000). Nonetheless, in contrast to

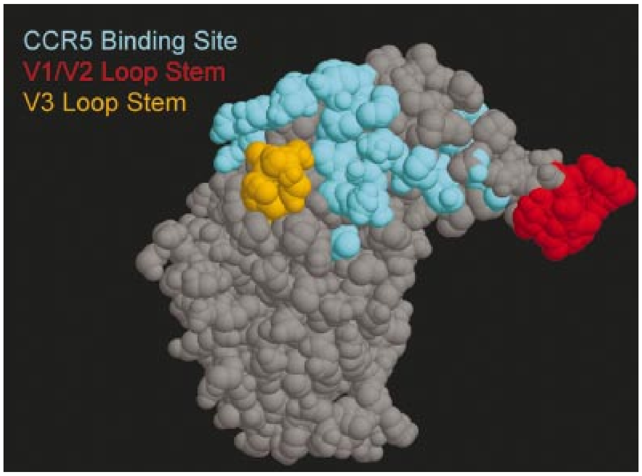

Fig. 2. Predicted coreceptor-binding site on HIV-2 gp120. Space-filling model of HIV-2 gp120 core indicating potential residues involved in coreceptor interactions and the location of the V1/V2 and V3 loop stems. This structure was predicted by SWISSPROT, based upon existing HIV-1 gp120 core crystals (pdb accession codes 1GC1, 1G9N and 1GM9) and rendered with RASMOL, version 2.7.1.

HIV-1, some HIV-2 and SIV strains are able to infect PBMCs independently of either CCR5 or CXCR4 (Chen *et al.*, 1997b, 1998; Simmons *et al.*, 2000; Sol *et al.*, 1997; Zhang *et al.*, 2000) implicating a potential role of at least some alternative receptor(s) for infection *in vivo*. Additionally, HIV-2 infection may result in higher levels of β chemokine production compared to HIV-1 infection (Akimoto *et al.*, 1998; Kokkotou *et al.*, 2000) and may thus drive evolution of alternative receptor use.

Coreceptor domains that are involved in mediating HIV-2 infection are similar to those required for HIV-1. For example, the N terminus and second extracellular loop of CXCR4 are important in mediating fusion and infection of HIV-2 ROD (Brelot *et al.*, 1997; Potempa *et al.*, 1997; Reeves *et al.*, 1998).

Virus tropism and coreceptor use *in vivo*

While all HIV-1, HIV-2 and SIV isolates infect primary CD4$^+$ T cells, isolates can differ in their relative tropism for macrophages and human T cells lines, as well as in their replication rates, and were thus historically divided into three groups. The first group consists of viruses that infect CD4$^+$ T cell lines *in vitro*, induce syncytia (multinucleated giant cells) and replicate to high titres relatively quickly and are termed syncytium-inducing, T cell tropic or rapid/high viruses. The use of CXCR4 (X4 tropism) is characteristic of these viruses. The second group consists of those that infect macrophage cultures efficiently, do not induce syncytia in infected primary T cells and replicate slowly to relatively low titres and are thus referred to as macrophage tropic, non-syncytium-inducing or slow/low viruses. These viruses typically use CCR5 as their major coreceptor and are referred to as R5 tropic. The third group can infect both T cell lines and macrophages and are thus referred to as dual-tropic viruses. These viruses typically use both CXCR4 and CCR5 and are termed R5/X4. Thus, virus tropism is largely explained by coreceptor usage, although a host of other factors, including receptor density, conformation and post-entry events, can also influence virus infection. Additionally, primary syncytium-inducing isolates can infect macrophages via CXCR4 (Simmons *et al.*, 1998).

For HIV-1, the importance of CCR5 for virus infection was shown by the discovery that individuals who lack CCR5 are highly resistant to virus infection (Liu *et al.*, 1996; Samson *et al.*, 1996). R5 virus strains are predominantly transmitted, are the major virus population in asymptomatic individuals and usually remain present throughout the course of infection (Connor *et al.*, 1997; de Roda Husman *et al.*, 1999; Huang *et al.*, 1996; Li *et al.*, 1999; van't Wout *et al.*, 1998). R5X4 viruses may precede the evolution to X4 tropism, which occurs in less than 50% of AIDS patients (Connor *et al.*, 1997; Tersmette *et al.*, 1988). An evolution from R5 to X4 is not obvious in HIV-2-infected individuals as many primary isolates use a range of coreceptors including both CCR5 and CXCR4 and only a limited number of X4 viruses have been isolated from symptomatic patients (Guillon *et al.*, 1998; Morner *et al.*, 1999; Reeves *et al.*, 1999). In addition, it is not known if R5 HIV-2 strains are largely responsible for virus transmission, as only a few isolates from asymptomatic individuals predominantly use CCR5 (Guillon *et al.*, 1998; Owen *et al.*, 1998).

CD4-independent virus infection

One of the most striking differences between HIV-1 and HIV-2 strains is that, while HIV-1 infection usually requires both CD4 and a coreceptor, many primary HIV-2 isolates exhibit some degree of CD4-independence (Reeves *et al.*, 1999). Even some HIV-2 strains that typically require CD4 to infect cells can be induced to infect CD4$^-$ cell lines by pretreatment with soluble CD4 (sCD4) (Clapham *et al.*, 1992; Reeves *et al.*, 1999). In addition, CD4-independent HIV-2 variants can arise spontaneously *in vitro* from chronically infected T cell lines (Clapham *et al.*, 1992; Endres *et al.*, 1996). These viruses primarily use CXCR4 for infection of CD4$^-$ cells but can also use other 7TM receptors independently of CD4, including CCR3, CCR5, CCR8, GPR1, GPR15 and STRL33 (Endres *et al.*, 1996; Lin *et al.*, 2001; Reeves *et al.*, 1997, 2000; Simmons *et al.*, 2000). SIV strains that can use CCR5, GPR15 or STRL33 for CD4-independent infection have also been identified (Edinger *et al.*, 1997, 1999; Reeves *et al.*, 1999, 2000; Schenten *et al.*, 1999), while only a few CD4-independent HIV-1 strains that were selected *in vitro* have been described (Dumonceaux *et al.*, 1998; Kolchinsky *et al.*, 1999; LaBranche *et al.*, 1999).

The Env determinants responsible for the CD4-independence of two HIV-2 strains (ROD/B and vcp) have been analysed in detail (Lin *et al.*, 2001; Reeves & Schulz, 1997). Only two mutations were required to confer a minimal CD4-independent phenotype to ROD/B, a variant of the prototypic CD4-dependent HIV-2 ROD/A strain, and a further two mutations conferred efficient CD4-independent infection (Reeves & Schulz, 1997). These same mutations increase the fusogenicity of the envelope protein as well as increasing the sensitivity of the envelope to undergo conformational changes induced by sCD4. The CD4-independence of the HIV-1 IIIB variant 8x is mediated by mutations in similar regions of envelope as those found in ROD/B (LaBranche *et al.*, 1999), although the CD4-independence of other viruses is conferred by different regions of *env* (Dumonceaux *et al.*, 1998; Kolchinsky *et al.*, 1999). It is therefore apparent that CD4-independence can be acquired by multiple mechanisms and that only a few amino acid changes are needed to confer CD4-independence.

The fact that HIV-2 and SIV strains more readily infect CD4$^-$ cells compared to HIV-1 strains may indicate that their coreceptor-binding site is at least partially exposed in the absence of CD4, enabling direct contact with a coreceptor (Fig. 3). CD4 binding by these strains is likely to modify the 7TM-binding site to increase the affinity of the Env–7TM interaction

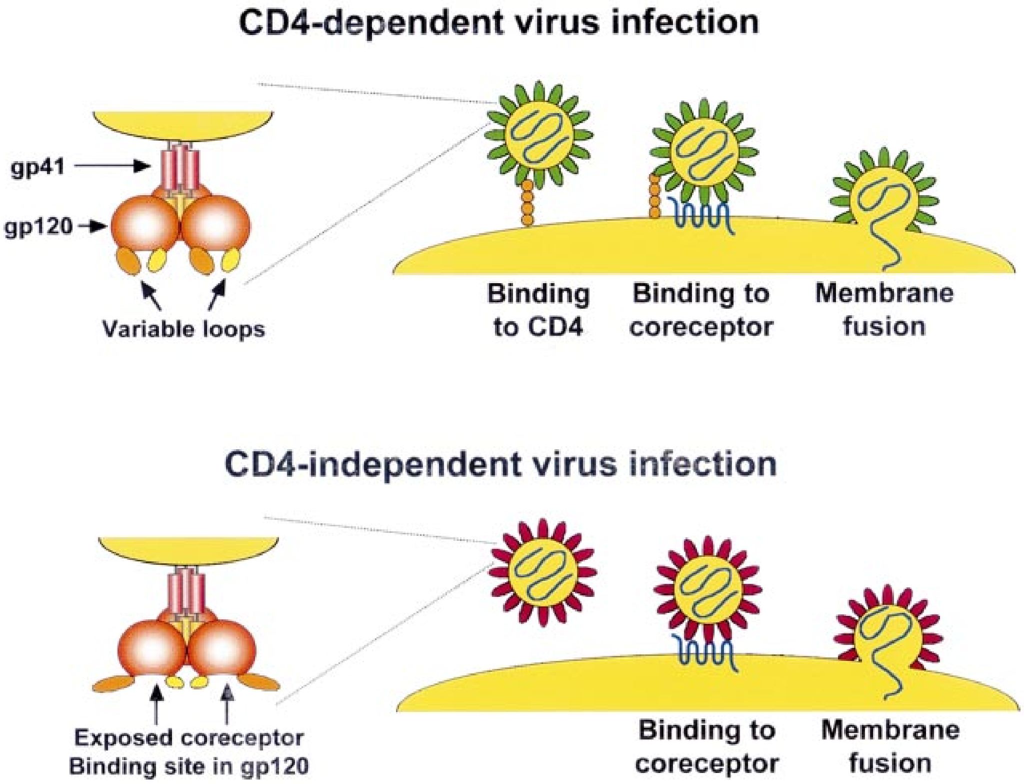

Fig. 3. CD4-dependent and CD4-independent infection. For CD4-dependent infection, gp120 first interacts with CD4 at the cell surface. This interaction induces conformational changes in Env that allow a secondary interaction with a 7TM coreceptor to occur. Following coreceptor binding, further conformational changes result in fusion of viral and cellular membranes. CD4-independent viruses can bypass CD4 and interact directly with a coreceptor molecule to initiate fusion. The ability to infect cells in the absence of CD4 is associated with HIV-2 and SIV strains and correlates with exposed coreceptor binding sites, broadened cell tropism and enhanced sensitivity to neutralization.

or to contribute extra energy to trigger fusion of virus and cell membranes. Either or both of these roles would provide HIV-2 with the capacity to exploit coreceptors that otherwise do not interact with gp120 strongly enough to trigger fusion. Indeed, CD4-independent HIV-2 strains that can use both CCR5 and CXCR4 to infect CD4$^+$ cells are only able to use one of these receptors in the absence of CD4, indicating that the use of CD4 enables a wider range of 7TM receptors to be exploited for infection (Reeves *et al.*, 1999).

Implications for pathogenesis

While CD4$^+$ haematopoietic cells are the major targets of HIV *in vivo*, infection of CD4$^-$ cells has been reported in a minority of HIV-1-positive individuals. However, the frequencies of infection of many CD4$^-$ cell types *in vivo* are unknown and there are no reports of HIV-2 tropism for CD4$^-$ cells *in vivo*. Nonetheless, CD4-independent viruses have the potential to infect a broader range of cell types *in vivo*. *In vitro* studies, coupled with the fact that viruses with distinct sequences and phenotypes can become compartmentalized in different tissues within an individual, such as the brain, lymphoid tissue and lungs (Epstein *et al.*, 1991; Itescu *et al.*, 1994; Sankale *et al.*, 1996), indicate that selection can occur at different sites *in vivo* and suggest some possible sites where CD4-independent viruses may be important. In the brain, for example, CD4$^-$ astrocytes are susceptible to HIV-1 infection *in vivo* (Bagasra *et al.*, 1996; Stoler *et al.*, 1986; Ward *et al.*, 1987; Wiley *et al.*, 1986), particularly in paediatric AIDS cases (Saito *et al.*, 1994; Tornatore *et al.*, 1994). Such infection is usually restrictive, however, with structural *gag* and *env* genes poorly expressed. HIV-2- and SIV-infected astrocytes have been cultured *ex vivo* from infected macaques, indicating that both HIV-2 and SIV can also infect astrocytes *in vivo* (Guillemin *et al.*, 1998). In addition, CD4-independent HIV-2 ROD/B can productively infect astrocytes *in vitro* via CXCR4 (Reeves *et al.*, 1999). A neurovirulent SIV strain can infect CD4$^-$ brain capillary endothelial cells (BCECs) both *in vitro* and *in vivo* (Edinger *et al.*, 1997; Mankowski *et al.*, 1994) and HIV-1 infection of BCECs has also been observed *in vitro* via CCR5 and *in vivo* (Bagasra *et al.*, 1996; Moses *et al.*, 1993; Ward *et al.*, 1987; Wiley *et al.*, 1986). Therefore, both HIV-2 and SIV strains have the potential to infect CD4-independently *in vivo* via an interaction with CCR5 or CXCR4.

Implications for vaccine design and therapy

HIV Env contains highly variable regions that may present antigenic decoys to the immune system. In addition, it is heavily glycosylated and attempts to generate broadly cross-reactive neutralizing antibodies have been largely unsuccessful. With the identification of the receptors used by HIV to infect cells coupled with structural studies, conserved, functionally important regions in Env have been identified that could be targets for neutralizing antibodies. Env proteins that elicit antibodies to conserved epitopes, such as the coreceptor-binding domain, may prove to be better vaccine candidates. CD4-independent virus strains can differ from CD4-dependent isolates by only a few amino acids, yet are invariably far more sensitive to neutralizing antibodies and HIV-positive human serum (Edwards *et al*., 2001; Hoffman *et al*., 1999). Therefore, the conformation of Env glycoproteins that allows a direct interaction with coreceptors may reveal epitopes to neutralizing antibodies that are usually exposed only after CD4 binding and thus favourably influence the capacity of the host to control virus replication. Monoclonal antibodies to such epitopes do interact preferentially with CD4-independent HIV-1 strains (Edwards *et al*., 2001; Hoffman *et al*., 1999). The very low virus load observed during asymptomatic HIV-2 infection may result partly from better immune control of viruses with 'open' Env proteins, which, in turn, could explain why many HIV-2-infected individuals often survive significantly longer than those infected with HIV-1. Indeed, sera from HIV-2-infected individuals are often broadly cross neutralizing (Bjorling *et al*., 1993; Robert-Guroff *et al*., 1992; Whittle *et al*., 1998). It will be important to determine if genetically triggered, CD4-independent Env proteins can preferentially elicit neutralizing antibodies in addition to being more susceptible to them.

Implications for virus evolution

The ability of many HIV-2 and SIV strains to infect cells independently of CD4 suggests that the virus ancestors of HIV and SIV may have originally used a 7TM receptor alone. Acquisition of a second receptor, i.e. CD4, may have provided selective advantages to a virus that persistently replicates in the face of a vigorous host immune response. On HIV-1 envelopes, the gp120 site for binding a 7TM receptor is only fully exposed following contact with CD4 (Figs 2 and 3). This mechanism may enable potential neutralizing epitopes on or around the 7TM-binding site to be hidden until the fusion reaction is triggered. Binding to CD4 also enables a wider range of 7TM receptors to be exploited for infection (Reeves *et al*., 1999) and may assist adaptation or switching to new coreceptors *in vivo*. Indeed for HIV-1 strains, CD4 use may aid the evolution from R5 to R5X4 tropism, which seems to compromise or weaken the interaction of Env with CCR5, as infection via CCR5 for R5X4 strains is especially sensitive both to CCR5 amino acid substitutions (Bieniasz *et al*., 1997; Picard *et al*., 1997) and to inhibition by RANTES (Kledal *et al*., 1997).

Concluding remarks

HIV-2 provides an interesting contrast to HIV-1. While HIV-1 has spread rapidly throughout the world, HIV-2 has remained more restricted in its distribution, being relatively rare outside of West Africa. For the most part, HIV-2 behaves similarly to HIV-1 *in vitro*, with its ability to frequently infect cells CD4-independently being a notable exception. Yet, *in vivo*, HIV-2 is clearly less pathogenic than HIV-1; it is transmitted less efficiently, establishes lower virus loads in asymptomatic infection and results in slower disease progression. HIV-2 therefore provides an important opportunity to study immune control and pathogenesis of lentivirus infections in humans. In effect, HIV-2 appears to be a mildly attenuated form of HIV-1, yet is clearly more pathogenic than SIV in its natural host, the sooty mangabey. Identifying the virus and host factors that account for this are likely to clarify the mechanisms that account for the pathogenic nature of HIV-1 and may help to identify immune factors responsible for the control of HIV-2 infection. This, in turn, could assist in HIV vaccine development. Increased study of primary HIV-2 strains as well as HIV-2-infected individuals will be needed to accomplish these goals.

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