Contents available at **PubMed**
www.aidsreviews.com

PERMANYER

AIDS Rev. 2016;18:44-53

HIV Interaction With Human Host: HIV-2 As a Model of a Less Virulent Infection

José Miguel Azevedo-Pereira and Quirina Santos-Costa

Host-Pathogen Interaction Unit, Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal; and Instituto de Medicina Molecular (IMM), Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

Abstract

HIV-1 and HIV-2 are the causal agents of AIDS. While similar in many ways, a significant amount of data suggests that HIV-2 is less virulent than HIV-1. In fact, HIV-2 infection is characterized by a longer asymptomatic stage and lower transmission rate, and the majority of HIV-2-infected patients can be classified as long-term non-progressors or elite controllers. The mechanisms underlying the ability of human host to naturally control HIV-2 infection are far from being completely understood. The identification of the differences between HIV-1 and HIV-2 interactions with human host cells could provide important insights into several aspects of retroviral pathogenesis that remain elusive, with significant implications for HIV vaccine development and therapy. In this review, we delve into some of the differences that notably distinguish HIV-2 from HIV-1, highlighting possible consequences in the pathogenesis and natural history of both infections. **(AIDS Rev. 2016;18:44-53)**

Corresponding author: José Miguel Azevedo-Pereira, miguel.pereira@ff.ul.pt

Key words

HIV-2. Chemokine receptor. CD4-independent. Intrinsically disordered protein. Envelope glycoprotein. V1/V2 region. Pathogenesis. Neutralization.

Introduction

Both HIV-1 and HIV-2 belong to the *Retroviridae* family, subfamily *Orthoretrovirinae*, genus *Lentivirus*. Accordingly, they share structural, antigenic, and genomic characteristics. However, despite both viruses being associated to the onset of AIDS, they show different pathogenic abilities in the human host (refer to table 1 for an overview of some differences between HIV-1 and HIV-2). In fact, HIV-2 infection is in general characterized by lower levels of viremia, lower transmission rates, and slower rates of disease progression compared to HIV-1 infection (reviewed[1]). Based on these virulence differences, we may consider the majority of HIV-2-infected patients as long-term non-progressors or elite controllers, which define a rare group of HIV-1-infected individuals who naturally maintain high $CD4^+$ T lymphocyte counts and undetectable viral loads for decades during the course of infection[2].

Several factors should be involved in the ability of human host to spontaneously control viremia and immunological failure during a pathogenic lentiviral infection such as HIV-2 infection. A better innate immune response mediated by dendritic cells could be triggered during initial events of infection, soon after transmission (reviewed[3]). Also, a more efficient adaptive immune response characterized by potent and broadly reactive neutralizing antibodies has been referred to as a hallmark of HIV-2-infected individuals[4-6]. However, the increased capacity to immunologically control HIV-2 infection should not be considered as an intrinsic characteristic of

Correspondence to:
José Miguel Azevedo-Pereira
Faculdade de Farmácia, Universidade de Lisboa
Av. Prof Gama Pinto
1649-003 Lisbon, Portugal
E-mail: miguel.pereira@ff.ul.pt

Table 1. Comparison of some HIV-1 and HIV-2 characteristics

		HIV-1	HIV-2
Epidemiology	Zoonotic origin	SIVcpz (*Pan troglodytes troglodytes)*	SIVsm (*Cercocebus atys)*
	Transmission	Sexual, blood-borne, and vertical transmission	Same routes of transmission, but less efficiently transmitted
	Geographic distribution	Worldwide	Restricted to West Africa and countries sharing socioeconomic links with them (e.g. Portugal and France)
Structure	Genome	Organized in prototypical (*gag, pol, env*), regulatory (*tat, rev*) and accessory genes (*vif, vpr, vpu, nef*)	Identical genomic organization. Yet HIV-2 lacks *vpu* and has an accessory *vpx* gene
	Viral particle	Enveloped; cone-shaped core; two genomic RNA molecules; virions 100-120 nm in diameter	Indistinguishable from HIV-1
Clinical features	Acute infection	High levels of viremia; reduction of $CD4^+$ lymphocytes; in some cases clinically characterized by a rash and a flu-like syndrome	No data regarding acute infection; probably similar to HIV-1
	Asymptomatic stage	Without treatment, usually lasts less than 10 years	Usually lasts decades
	AIDS	Characterized by low CD4 counts and the emergence of opportunistic infections and tumors	After the onset of AIDS, it is characterized by a similar set of clinical features as HIV-1
	Viral load	Usually moderate to high, depending on the disease stage	Usually undetectable, except during full-blown AIDS
Cell receptors interaction	Coreceptor usage	Mainly CCR5 and CXCR4; alternate coreceptors rarely reported	Efficient use of many different coreceptors besides CCR5 and CXCR4 (e.g. CCR8)
	CD4-independent infection	Rarely described in primary isolates	Several primary isolates reported as being able to infect CD4-negative cells *in vitro*
	Determinants of coreceptor usage	Mainly V3 region of SU glycoprotein	Conflicting data. Mainly V1/V2 region of SU glycoprotein and, to a lesser extent, V3 region

SIV: simian immunodeficiency virus. SU: surface.

infected patients, but rather a consequence of a less competent lentivirus. In other words, the lesser virulence of HIV-2 may derive from viral vulnerabilities that lead to a lesser replicative capacity and to a better host immune response.

One of the factors that may account for those additional vulnerabilities is the interaction of HIV-2 with cell receptors during the early steps of the replication cycle. In this review, we provide data regarding the differences in coreceptor engagement between HIV-1 and HIV-2 and the potential implications of those differences in viral pathogenesis, underlining how important and helpful a deeper understanding of HIV-2-cell interactions may be for future HIV vaccine design and therapy.

The disease, the viruses and their epidemiology

The first virus associated with AIDS was identified in 1983[7] and is now known as HIV-1. A few years later a second related virus was isolated: HIV-2[8]. Both HIV-1 and HIV-2 were introduced into the human population as a consequence of multiple zoonotic events leading to cross-species transmission from simian immunodeficiency virus (SIV)-infected non-human primates. Genetic and epidemiological analysis of HIV-1, HIV-2, and SIV strains allowed the identification of SIVcpz (infecting the chimpanzee subspecies *Pan troglodytes troglodytes*) and SIVsm (infecting sooty mangabey subspecies *Cercocebus atys*)

as viral ancestors of HIV-1 and HIV-2, respectively[9,10]. Apparently, several cross-species events were observed, each of them giving rise to a new group within HIV-1 and HIV-2 (reviewed[11]). These events were then fuelled-up by distinct socioeconomic-nosocomial factors that provided the optimal conditions for dissemination of these blood-borne and sexually transmitted viruses[11].

Although sharing identical transmission routes, the epidemiology of HIV-1 and HIV-2 shows clear distinct patterns: while the global HIV epidemic is dominated by HIV-1, particularly group M, HIV-2 has remained restricted largely to West Africa and countries that shared socioeconomic links with this region (e.g. Portugal, France, and their former colonies). The limited expansion of HIV-2 should be related with less efficient transmission between human hosts. In fact, it was reported that HIV-2 is five to nine times less efficiently transmitted than HIV-1 by sexual route[12]. Similarly, the vertical transmission rate of HIV-2 is 0-4%, while in HIV-1 this transmission occurs in 15-40% of untreated pregnancies[13].

Considering that HIV is transmitted mainly through unprotected sexual intercourse, several factors are involved, dictating a successful transmission. Some are related with mucosal barrier and the interactions that HIV establishes with cells present in cervical/vaginal/foreskin/anal mucosa epithelial surfaces[14], particularly with antigen presenting cells (APC) that populate the sub-epithelial layer of mucosal tissue (e.g. dendritic cells and macrophages). In this regard, little is known about the efficiency with which HIV-2 interacts with APCs or the fate of viral particles during and after interaction with cell receptors present in those cells (e.g. DC-SIGN, DCIR, CD169, CCR5, CD4). Considering the reduced rate of sexual transmission, a detailed understanding of the HIV-2 interaction with these cells may help the identification of additional vulnerabilities during sexual transmission, providing important clues to its reduced spread.

The infection of dendritic cells (DC) itself could also explain some of the differences in pathogenesis observed between HIV-1 and HIV-2 (reviewed[3]). In fact, HIV-1 and HIV-2 are differently affected by SAMHD1, a specific restriction factor that impairs reverse transcription leading to non-productive infection of DCs. While HIV-2 counteracts SAMHD1 restriction through viral protein Vpx, replication of HIV-1 (that lacks Vpx) is suppressed by SAMHD1. This results in a lower HIV-1 replication in DCs, which may enable HIV-1 avoidance of interferon-mediated antiviral immunity. Therefore, while SAMHD1 renders DCs less permissive to HIV-1 infection, it seems also responsible for the HIV-1 evasion of immune sensing. In contrast, HIV-2 due to the presence of Vpx, is able to replicate in DCs, leading to a positive effect in the innate sensing and in the adequate priming of adaptive immunity[3].

HIV and cellular coreceptors: the chemokine receptors connection

Although HIV isolation, its causative relation to AIDS, and the main structural, genomic, and replicative characteristics were reported more than three decades ago, the intimate relation between HIV and the chemokine system has remained illusory for many years. In the 1990s, two separate sets of observations demonstrated that chemokines and their respective receptors had crucial roles in HIV-1 infection. First, the identification of chemokines MIP-1α, MIP-1β (macrophage inflammatory protein-1 alpha and beta, respectively) and RANTES (regulated on activation normal T-cell expressed and secreted) as the major components of $CD8^+$ T lymphocytes soluble factor that strongly inhibited HIV replication[15]. These proteins are members of the CC family of chemokines and this observation has shed light in the non-cytotoxic mechanism by which $CD8^+$ T lymphocytes suppress HIV replication[16]. The next major observation was the identification of a second HIV cellular receptor (coreceptor) that in addition to CD4 must be present at cell membrane to allow virus entry. This coreceptor was shown to be a chemokine receptor: Fusin, later named CXCR4[17]. Soon after this first coreceptor was identified, a second chemokine receptor (CCR5) was also associated with HIV entry[18], and a natural 32-bp deletion within the *ccr5* gene was related with resistance to HIV infection[19].

All these findings have had a major impact on the understanding of HIV pathogenesis and in the mechanisms of viral entry. In this regard, it is now accepted that to trigger HIV entry, the surface (SU) envelope (Env) glycoprotein must sequentially engage the CD4 receptor and the coreceptor (such as CCR5 or CXCR4). This two-step receptor interaction allows HIV to hide the highly conserved coreceptor binding site from neutralizing antibodies, unraveling it only after SU-CD4 interaction and when viral envelope and cell membrane are in close contact. Binding of SU to coreceptor molecule induces further conformational changes that allow the disclosure of an amino-terminal hydrophobic region (fusion peptide) of the transmembrane (TM) Env glycoprotein subunit. The insertion of the fusion peptide into the cell surface leads to the fusion of viral envelope with the cell membrane and the release of viral nucleocapsid into the cytoplasm (reviewed[20]).

Table 2. Consequences in viral pathogenesis of some HIV-2 characteristics

Characteristic of HIV-2 infection	Expected consequences in the pathogenesis
Interaction with other coreceptors besides CCR5 and CXCR4	Inadequate intracellular signaling, an event that seems to be required for productive infection. Additionally, these interactions could lead to the infection of non-activated cells and to abortive replication cycle. Both mechanisms will lead to a decrease in viral production and to a lesser viral load.
Avoidance of SAMHD1 inhibition in dendritic cells	Replication in dendritic cells; triggers innate sensing mechanisms leading to interferon-mediated antiviral immunity; adequate priming of adaptive immunity. Both mechanisms may lead to a better immunological control of HIV-2 infection.
Lower viral load	Lower transmission rates (blood-borne, sexual, or mother-to-child). Decreased T-cell depletion?
CD4-independent infection	Direct interaction with coreceptor imposes the pre-exposure or preformation of the coreceptor binding site. This conformation of surface glycoprotein might elicit neutralizing antibodies targeting the critical coreceptor binding step, favoring the host immunological control of HIV-2 infection.

After the initial identification of CXCR4 and CCR5, several other chemokine receptors have been described as being able to act *in vitro* as coreceptors for HIV-1, HIV-2, and SIV. The ability of different HIV isolates to use distinct coreceptors revealed a major difference between HIV-1 and HIV-2. In fact, early reports had clearly shown that while in HIV-1 only CCR5 and CXCR4 appear to be the major coreceptors, in HIV-2 many other chemokine receptors could be engaged *in vitro* as efficiently as CCR5 or CXCR4[21-23].

HIV-2: a "different" HIV

As mentioned, although sharing identical structural and genomic properties, HIV-1 and HIV-2 show different pathogenic abilities in human host. Typically, HIV-1 infection is characterized by a gradual and irreversible depletion of $CD4^+$ T lymphocytes, which leads within a median time of 10 years to immune dysfunction and the development of AIDS. However, in contrast to this typical progression, virtually all HIV-2 and a small percentage[2] of HIV-1 infections (referred to as "long-term non-progressors" or "elite controllers") remain healthy for several decades, with undetectable plasma viral load, $CD4^+$ T lymphocyte counts above 500 cells/μl, and a longer asymptomatic stage and slower progression to AIDS[24-26]. Some studies even show that HIV-2 infection has no effect on survival in adults[25,26] and apparently it could offer some kind of protection if a subsequent HIV-1 infection occurs, lowering the rate of disease progression and mortality in dual-infected patients compared to HIV-1 mono-infected[27].

All these findings suggest that in HIV-2 infection, in contrast to HIV-1, a distinct equilibrium between virologic and immunologic factors should facilitate a best-fitted immune response, leading to better control of HIV-2 infection (Table 2). The disclosure of the factors underlying the delayed disease progression observed in HIV-2-infected patients may help clarify AIDS pathogenesis and assist in the identification of correlates of protection crucial for the development of an effective HIV vaccine.

One of the factors that may shape the lesser virulence of HIV-2 infection is the way it interacts with cellular receptors. In fact, several reports indicate that the two types of HIV engage these receptors in different ways, which may affect viral fitness. Particularly, the interactions with coreceptor molecules appear to have structural requirements that clearly distinguish them. In addition to the aforementioned broader usage of chemokine receptors as cofactors for viral entry, HIV-2 isolates able to directly interact with coreceptor without prior binding to CD4[28,29] and the identification of CCR5-/CXCR4-independent isolates[30-32] have created the notion that during HIV-2 entry, distinct structural mechanisms govern this critical step of the viral replication cycle.

As referred, binding of HIV SU glycoprotein to CD4 allows the initiation of the fusion process. Interestingly, for most HIV-1 strains, the SU glycoprotein interaction with CD4 is of higher affinity when compared with HIV-2[33]. Deglycosylated SU glycoprotein retains a significant capacity to bind to CD4, indicating that carbohydrate chains of HIV-2, as those of HIV-1, do not play a central role in the SU-CD4 interaction[34]. Nonetheless, carbohydrate chain is necessary for a correct folding of SU glycoprotein to provide a CD4-binding site, and removal of potential glycosylation residues drastically affects the efficiency of HIV-2 SU binding to CD4[35]. In addition, HIV-2 has a capacity to infect cells independently of CD4 receptor. Several primary HIV-2 isolates have been identified as being

able to directly interact with coreceptor without prior binding to CD4 molecule[28,29,36], suggesting that high levels of CD4 expression are not critical for HIV-2 entry (at least for some isolates), contrasting with HIV-1 infection where the identification of primary isolates able to infect cells in the absence of CD4 receptor is rare.

The ability to infect CD4-negative cells could be seen as an advantage for HIV-2 since this feature enables the infection of a broader range of cell types (e.g. CD4 negative/coreceptor positive cells or cells with low CD4 expression) located in different body compartments such as the brain, testes, liver, lymphoid tissue, kidneys, or lungs. However, considering the accepted mechanism for HIV entry into target cells, it also implies that the coreceptor binding site must be totally or partially exposed, or formed, prior to virus attachment to cell membrane[37]. If CD4-independent isolates exist *in vivo,* this feature may result in premature exposure of epitopes that could elicit neutralizing antibodies targeting the coreceptor binding site, thus favoring host immunological response. In turn, this may help explain the existence of sera from HIV-2-infected individuals showing a potent and broad neutralizing activity[4,5]. Accordingly and sustaining this hypothesis, monoclonal antibodies targeting epitopes within this region of SU glycoprotein are much more efficient against CD4-independent isolates than to CD4-dependent counterparts[37,38].

The existence of an immunological-driven counter selection as described above may be responsible for the infrequent detection of CD4-independent HIV-1 isolates. Interestingly, CD4-independent infection of some HIV strains seems to involve an endocytic route, involving endosomal acidification and cathepsin protease activity (e.g. cathepsin B)[39]. In the absence of CD4 interaction, the digestion by cathepsin proteases may be required to convert Env SU glycoprotein to a fusion-active form as observed for murine leukemia virus and Ebola virus[40-42]. However, this entry pathway by CD4-independent HIV isolates also exposes internalized virions to degradation by cathepsins in late endosomal compartments, leading to a competition between fusion, required for productive infection, and degradation that destroys the incoming viral particles (reviewed[43]).

Chemokine receptor usage by HIV-1 strains has been generally described as a tale of two heroes: HIV-1 either uses CCR5 or CXCR4 (and sometimes both). Apparently CCR5 coreceptor is used by isolates (dubbed R5 variants) obtained soon after transmission and during the chronic asymptomatic stage of infection[44]. The CXCR4 usage may be acquired during disease progression as a result of viral evolution and adaptation to different host-driven constrains. These strains (referred as X4 variants), characterized by a greater cytopathic capacity and an increased replication rate, will eventually predominate in approximately half of the patients in late disease stages, usually linked to accelerated $CD4^+$ depletion and disease progression[45]. Interestingly, a study addressing coreceptor usage by a large cohort of HIV-1 and HIV-2 isolates revealed that the emergence of X4 variants is more common in HIV-1 than in HIV-2[46]. Due to the described characteristics of these variants, it is conceivable that the predominance of non-X4 viruses in HIV-2 may help the preservation of $CD4^+$ lymphocytes repertoire and immune system functionality, in contrast to HIV-1 where the more frequent detection of X4 variants may lead to increased cell depletion and limited T-cell regeneration[47,48]. However, despite the major role of CCR5 and CXCR4 in HIV-1 pathogenesis, other chemokine receptors (described as "alternate" or "minor") may also contribute to HIV-1 infection in some cell types or in some circumstances. For example, in spite of the importance of R5 viruses during HIV transmission[49], a recent report has revealed a transmitted-founder HIV-1 able to use three different coreceptors instead of CCR5 or CXCR4 (GPR-15, APJ, and FPRL-1[50]), underlining the notion that unusual coreceptors may be used in some circumstances by HIV-1 *in vivo*.

In contrast to the almost clear dichotomy scenario in HIV-1 (i.e. CCR5 vs. CXCR4), it has been widely shown that primary HIV-2 isolates have in general a broader coreceptor usage than HIV-1 (reviewed[1,51]). It is noteworthy that some HIV-2 isolates are able to exploit these alternate coreceptors *in vitro* as efficiently as CCR5 or CXCR4, even those rarely described for HIV-1. For instance, it was recently demonstrated that some primary HIV-2 isolates are unable to use CCR5 or CXCR4; instead they enter cells using CCR8 as coreceptor[52]. The role of these alternate coreceptors *in vivo* is far from being clarified and very few data exist regarding which coreceptors are used during the course of virus-host interplay. It should be also noted that although the interactions with alternate coreceptor could mediate viral entry, they might be insufficient to elicit the appropriate intracellular signaling that is required for productive infection in primary cells[53]. Additionally, they could lead to the infection of cell populations that are not activated and consequently not permissive to a productive HIV infection[54].

The variety of distinct possibilities regarding coreceptor usage by HIV-1, and particularly HIV-2, reinforces the concept that *in vivo,* the initial virus-cell interactions are governed by a complex interplay between cell membrane-bound molecules and highly flexible and variable

viral proteins. Notably, HIV Env SU glycoprotein belongs to a large universe of proteins known as intrinsically disordered proteins (IDP) or proteins with intrinsically disordered regions (IDR). Such proteins and regions, although biologically active, fail to form specific structures, existing instead as dynamic conformational ensembles (recently reviewed[55]). Characteristically, viruses have several IDPs and proteins with IDRs. A comparative study between proteomes from different viruses, bacteria, archaea, and eukaryotes revealed that viral proteins have a higher propensity for intrinsic disorder[56]. Apparently, this characteristic was driven by the need to maintain protein functionality despite the existence of compact/minimalist genomes and high mutation rates, the latter particularly evident in RNA-genome viruses. The presence of IDR within SU glycoprotein may influence the dynamic behavior of this protein and thus be responsible for the conformational diversity and structural plasticity shown by SU[57]. Both features play a crucial role in immune evasion (see below) and in HIV-cell receptor interactions, enabling HIV to interact with different coreceptors, depending on cell type or body compartment.

Molecular basis of coreceptor interaction and implications on HIV pathogenesis

The molecular determinants of SU glycoprotein dictating which coreceptor is used seem also to be different in HIV-1 and HIV-2. Soon after the discovery of coreceptors, the variable region 3 (V3) of HIV-1 envelope SU glycoprotein was suggested as the major structural determinant of SU-coreceptor interaction[58,59]. The amino acids involved in CCR5 and/or CXCR4 usage[60] are located mainly in the base and the stem of the V3 loop (Fig. 1). However, such relatedness was scarcely proved in HIV-2. Indeed, although some studies had claimed an association between different coreceptor usage and specific sequence motifs within the V3 region[61-64], others have failed to link the V3 amino acid sequence with coreceptor choice, reporting that no singular genetic signature could be proposed to explain different coreceptor usage[31,65,66]. Instead, a recent study has disclosed a critical role of the adjacent variable regions 1 and 2 (V1/V2) in CCR5 (R5), CXCR4 (X4) and CCR8 (R8) usage by HIV-2[52]. Using a panel of isogenic mutant viruses, it was demonstrated that the switch from R8 to R5 or R8 to R5X4 phenotype is determined by amino acids located in the base and tip of V1/V2 loops (Fig. 2).

How V1/V2 region interacts with cell coreceptors, how different Env structures are spatially organized, or which conformational changes they must undergo during receptor/coreceptor binding remain essentially unknown in HIV-2. From data regarding the structure of HIV-1 Env glycoproteins, it seems clear that although V1/V2 is dispensable for viral entry, it is crucial to escape antibody mediated neutralization[67-70]. This protective role of the V1/V2 region has been related to two different mechanisms: the first derives from the intrinsic characteristics of this region, namely its remarkable antigenic variation, the presence of several putative glycosylation sites, and the length variation of this region; the second is related to structural interactions within Env glycoprotein where the V1/V2 region plays a major role in conformational masking, protecting neutralization-sensitive domains either in the same SU glycoprotein or in adjacent subunits in the context of the Env complex[70]. These neutralization-sensitive domains include: (i) the V3 loop as a crucial component, together with V1/V2 itself, in the formation of coreceptor binding-site; (ii) the CD4 binding site; and (iii) CD4-induced epitopes.

In HIV-2, uncertainties prevail as to which structural interactions and conformational dynamics should exist between different domains of trimeric Env complexes[52]. Could the data acquired from the HIV-1 model also be applicable to HIV-2? To what extent are mechanisms disclosed for HIV-1 also dictating the tertiary and quaternary structure of HIV-2 envelope glycoproteins? Are there significant differences in the role of IDR in spatial organization and/or flexibility of HIV-1 and HIV-2 SU glycoproteins? Some facts are important to remember at this point. One is that sera obtained from HIV-2-infected individuals show potent and broad neutralization capacity to both autologous and heterologous isolates[4,5,27]. This fact is surely related to the lower viral load and increased survival observed in HIV-2-infected individuals. This fact also reveals that HIV-2 may have specific vulnerabilities that make it less able to evade neutralizing antibodies. The aforementioned capacity of HIV-2 to efficiently interact with different coreceptors, and the natural occurrence of CD4-independent variants, suggest a more open native conformation of HIV-2 SU glycoprotein, facilitating the neutralization of critical domains by host antibodies. Therefore, the mechanisms underlying the natural elicitation of broadly neutralizing antibodies in HIV-2-infected patients must be further understood in order to better inform rational HIV-1 vaccine design.

Another important aspect regarding HIV-2 neutralization is that V3 region, together with flanking regions C2 and C3, seems to be much less important as a target for host neutralizing antibodies[71], in contrast to what is observed in HIV-1. Inversely and worth noting, the V1/V2 region has long been described as a target for neutralizing

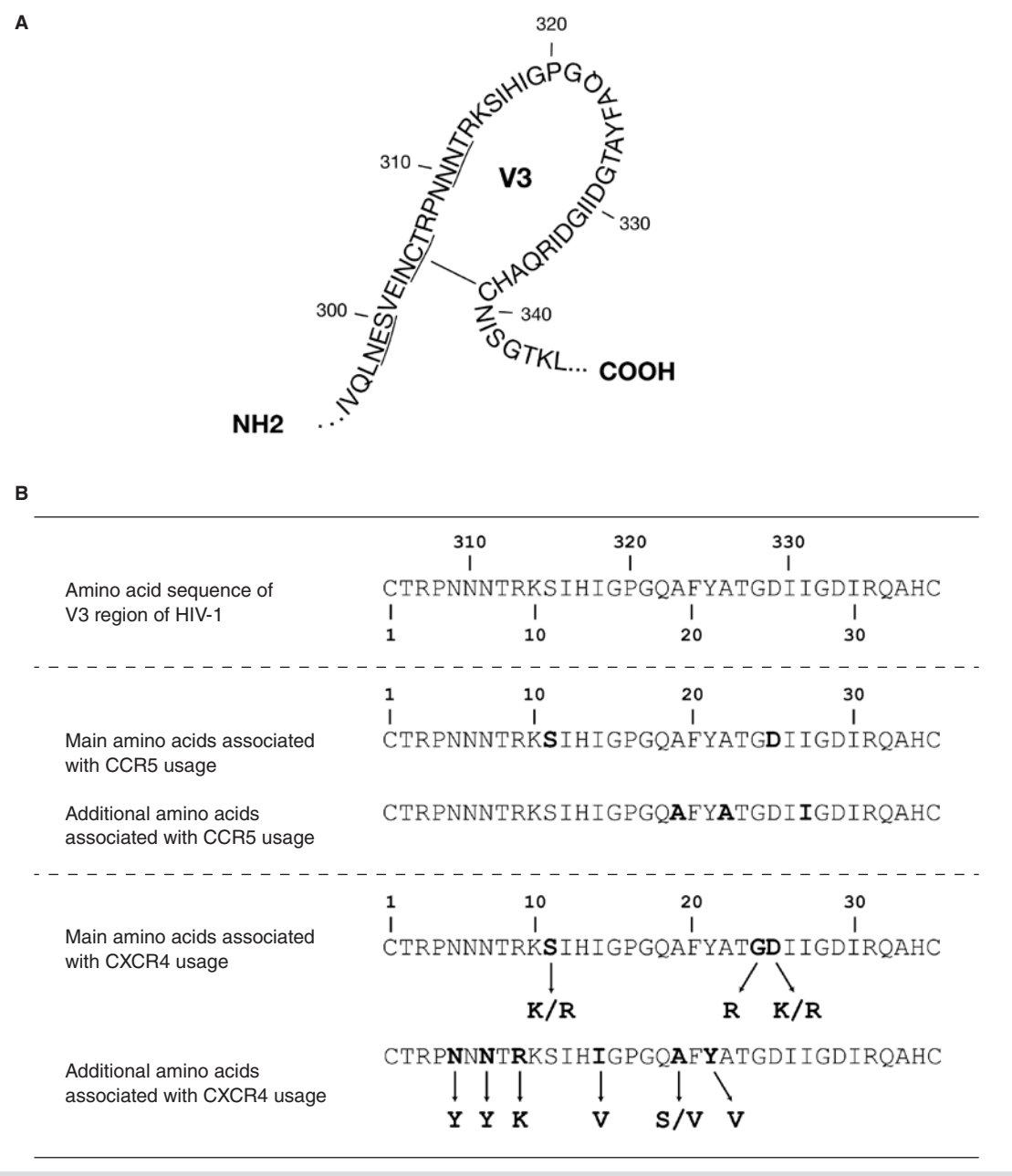
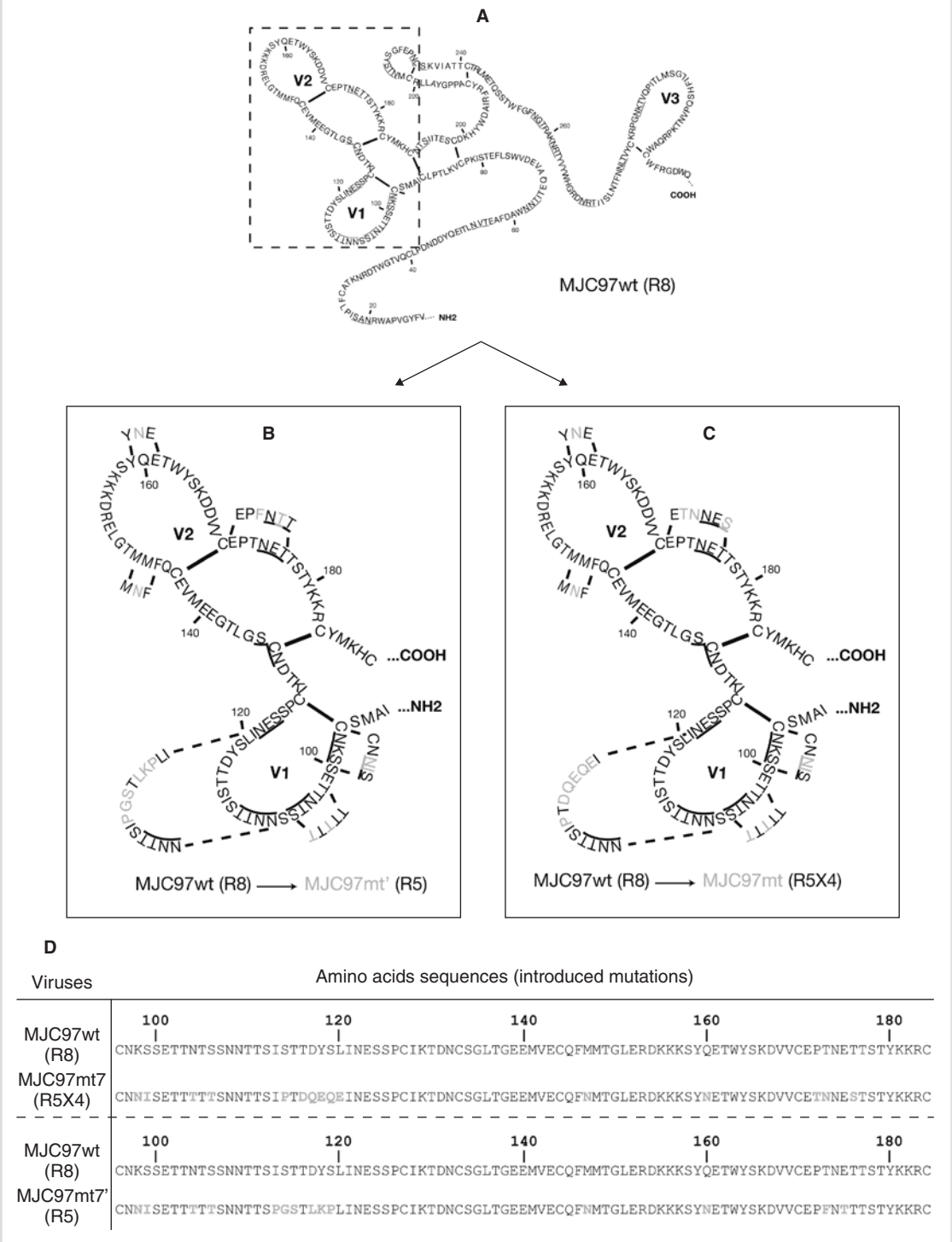


Figure 1. *Amino acids involved in CCR5 and CXCR4 usage by HIV-1. Panel **A** is a schematic representation of the amino acids present in the consensus sequence of HIV-1 isolates. Those amino acids determining CCR5 or CXCR4 usage are indicated in panel **B** in bold type, differentiated in major and minor determinants60. Amino acids are denoted by single-letter code. The putative N-linked glycosylation sites are identified in panel **A** by underlined amino acids. Amino acid residues are numbered according to consensus sequence of HIV-1 (http://www.hiv.lanl.gov/content/index). The 35 amino acids of the V3 region sequence are also numbered beginning in the first cysteine (denoted as 1).*

monoclonal antibodies *in vitro*, and the overall conformation of this region seems to affect the sensitivity to neutralization[72]. Furthermore, a recent vaccine efficacy trial against HIV-1 (the RV144 trial) revealed that V1/V2 region elicits host-neutralizing antibodies *in vivo*[73].

The intersection of the previous paragraphs may lead us to interesting suggestions. If we assume that V1/V2 region of HIV-2 envelope also elicits host-neutralizing antibodies (as for HIV-1 in the RV144 vaccine trial), and if this region is simultaneously a determinant of coreceptor



D

Viruses	Amino acids sequences (introduced mutations)
	100 120 140 160 180
MJC97wt (R8)	CNKSSETTNTSSNNTTSISTTDYSLINESSPCIKTDNCSGLTGEEMVECQFMMTGLERDKKKSYQETWYSKDVVCEPTNETTSTYKKRC
MJC97mt7 (R5X4)	CNNISETTTTTSNNTTSIPTDQEQEINESSPCIKTDNCSGLTGEEMVECQFNMTGLERDKKKSYNETWYSKDVVCETNNESTSTYKKRC
	100 120 140 160 180
MJC97wt (R8)	CNKSSETTNTSSNNTTSISTTDYSLINESSPCIKTDNCSGLTGEEMVECQFMMTGLERDKKKSYQETWYSKDVVCEPTNETTSTYKKRC
MJC97mt7' (R5)	CNNISETTTTTSNNTTSPGSTLKPLINESSPCIKTDNCSGLTGEEMVECQFNMTGLERDKKKSYNETWYSKDVVCEPFNTTTSTYKKRC

Figure 2. *Amino acids involved in coreceptor (CCR5, CCR8 and CXCR4) usage by HIV-2. Schematic representation of the envelope SU glycoprotein of HIV-2MJC97 putative secondary structure spanning from C1 to V3 regions. The amino acid sequence of HIV-2MJC97 (MJC97wt; panel **A**) are represented in black; the mutated amino acids present in MJC97mt7' (R5; panel **B**) and MJC97mt7 (R5X4; panel **C**) are represented in red. The V1V2 primary amino acid sequences of both MJC97wt and mutated variants (MJC97mt7 and MJC97mt7') are also represented in panel **D**. Amino acids are denoted by single-letter code. The underlined amino acids in panels **A**, **B**, and **C** represent potential glycosylation sites linked to asparagine (N). Amino acid residues were numbered according to HIV-2MJC97 sequence (GenBank accession number: EU021092)* (adapted with permission from Santos-Costa, et al.[52] [original publisher: BioMed Central]).

engagement[52], this could constitute a major disadvantage for HIV-2 replication since the presence of antibodies directed to V1/V2 region will efficiently block HIV-2 entry. The differences between HIV-1 and HIV-2 regarding the structures involved in coreceptor binding and antibody neutralization may be analyzed in an overall perspective in which HIV-1 has evolved in order to achieve and accommodate two opposing requirements, both of which are directly linked to the exposed SU glycoprotein: the need to escape host's immune system pressure and surveillance, while preserving the functionality of envelope glycoproteins as specialized viral molecules to surpass the cell membrane barrier[74]. While the first drives a permanent change of immunodominant regions, the second requirement imposes the maintenance of crucial domains involved in receptor/coreceptor binding, thereby assuring the fusion between viral and cell membrane. The easiest way to conciliate these two requirements is by structural segregation of each domain. Therefore, in those domains more exposed to immune control, a high degree of genetic variability is allowed, while those involved in structural interactions with cellular receptors remain basically unchanged. Apparently, in HIV-2 this structural segregation between antigenically variable domains (V1/V2 region) and invariable functional domains (again the V1/V2 region), determining the interaction with cellular coreceptor, may be much less preserved, with expected implications on viral fitness.

In conclusion, the equilibrium that is established in HIV-2-infected patients between host's immune response and a less fitted viral population should provide an explanation for the longer survival and absence of disease onset. Nevertheless, HIV-2 infection eventually will lead to immunodeficiency and AIDS, probably as the result of selection of viral variants resistant to neutralizing antibodies, as suggested by the strong association between neutralization-resistance, advanced stages of HIV infection, and the predominance of CXCR4-using isolates within patient's viral population[75]. The mutations enabling the use of CXCR4 as coreceptor may provide the structural and conformational dynamics that ultimately facilitate the emergence of a more fit viral population. In general, this will only occur in late disease stages, providing another plausible explanation for the unusually long asymptomatic and aviremic course of HIV-2 infection.

Conclusion

Throughout this review, important aspects of the HIV interaction with cellular receptors have been pointed out. In all of them a common notion prevails: the differences observed between HIV-1 and HIV-2 pathogenesis are, at least in part, directly linked to early virus-cell interactions, more precisely during Env SU glycoprotein engagement of cell receptors. Despite the highly complex network of interconnected processes underlying HIV pathogenic mechanisms, the events that are triggered during and after virus binding to CD4 and the coreceptor have direct and crucial implications in the subsequent evolution of the host-pathogen interplay.

This review also highlights some mechanisms and molecular pathways that need to be further analyzed and better understood. Some of them are based on the lack of suitable *in vivo* models mimicking human host/HIV interactions. Nevertheless, we stress the appropriateness of studies aiming at HIV-2-cell interactions as a model to perceive the mechanisms enabling the control of a lentiviral infection for extended periods of time, *en route* to assisting the development of effective HIV vaccine and therapeutics.

Declaration of interest

This work was supported by grants from Fundação para a Ciência e Tecnologia and Ministério da Saúde de Portugal (FCT grant: VIH/SAU/0006/2011).

References

1. Azevedo-Pereira JM, Santos-Costa Q, Moniz-Pereira J. HIV-2 infection and chemokine receptors usage - clues to reduced virulence of HIV-2. Curr HIV Res. 2005;3:3-16.
2. Lambotte O, Boufassa F, Madec Y, et al. HIV controllers: a homogeneous group of HIV-1-infected patients with spontaneous control of viral replication. Clin Infect Dis. 2005;41:1053-6.
3. Barroca P, Calado M, Azevedo-Pereira JM. HIV/dendritic cell interaction: consequences in the pathogenesis of HIV infection. AIDS Rev. 2014;16:223-35.
4. Kong R, Li H, Bibollet-Ruche F, et al. Broad and potent neutralizing antibody responses elicited in natural HIV-2 infection. J Virol. 2012;86:947-60.
5. Ozkaya Sahin G, Holmgren B, da Silva ZJ, et al. Potent intratype neutralizing activity distinguishes human immunodeficiency virus type 2 (HIV-2) from HIV-1. J Virol. 2012;86:961-71.
6. De Silva TI, Aasa-Chapman MM, Cotten M, et al. Potent autologous and heterologous neutralizing antibody responses occur in HIV-2 infection across a broad range of infection outcomes. J Virol. 2012;86:930-46.
7. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science. 1983;220:868-71.
8. Clavel F, Guétard D, Brun-Vezinet F, et al. Isolation of a new human retrovirus from West African patients with AIDS. Science. 1986;233:343-6.
9. Keele BF, Van Heuverswyn F, Li Y, et al. Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. Science. 2006;313:523-6.
10. Lemey P, Pybus OG, Wang B, et al. Tracing the origin and history of the HIV-2 epidemic. Proc Natl Acad Sci USA. 2003;100:6588-92.
11. Sharp PM, Hahn BH. The evolution of HIV-1 and the origin of AIDS. Philos Trans R Soc Lond B Biol Sci. 2010;365:2487-94.
12. Kanki P, Mboup S, Marlink R, et al. Prevalence and risk determinants of human immunodeficiency virus type 2 (HIV-2) and human immunodeficiency virus type 1 (HIV-1) in West African female prostitutes. Am J Epidemiol. 1992;136:895-907.
13. Burgard M, Jasseron C, Matheron S, et al. Mother-to-child transmission of HIV-2 infection from 1986 to 2007 in the ANRS French Perinatal Cohort EPF-CO1. Clin Infect Dis. 2010;51:833-43.
14. Haase AT. Early events in sexual transmission of HIV and SIV and opportunities for interventions. Annu Rev Med. 2011;62:127-39.
15. Cocchi F, DeVico AL, Garzino-Demo A, et al. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. Science. 1995;270:1811-15.
16. Walker CM, Moody DJ, Stites DP, et al. CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. Science. 1986;234:1563-6.

17. Feng Y, Broder CC, Kennedy PE, et al. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science. 1996;272:872-7.
18. Alkhatib G, Combadiere C, Broder CC, et al. CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. Science. 1996;272:1955-8.
19. Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. Science. 1996;273:1856-62.
20. Clapham PR, McKnight A. Cell surface receptors, virus entry and tropism of primate lentiviruses. J Gen Virol. 2002;83:1809-29.
21. Bron R, Klasse PJ, Wilkinson D, et al. Promiscuous use of CC and CXC chemokine receptors in cell-to-cell fusion mediated by a human immunodeficiency virus type 2 envelope protein. J Virol. 1997;71:8405-15.
22. Guillon C, van der Ende ME, Boers PHM, et al. Coreceptor usage of human immunodeficiency virus type 2 primary isolates and biological clones is broad and does not correlate with their syncytium-inducing capacities. J Virol. 1998;72:6260-3.
23. McKnight A, Dittmar MT, Moniz-Pereira J, et al. A broad range of chemokine receptors are used by primary isolates of human immunodeficiency virus type 2 as coreceptors with CD4. J Virol. 1998;72:4065-71.
24. Whittle H, Morris J, Todd J, et al. HIV-2-infected patients survive longer than HIV-1-infected patients. AIDS. 1994;8:1617-20.
25. Poulsen AG, Aaby P, Larsen O, et al. 9-year HIV-2-associated mortality in an urban community in Bissau, West Africa. Lancet. 1997;349:911-14.
26. Marlink R, Kanki P, Thior I, et al. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science. 1994;265:1587-90.
27. Esbjornsson J, Mansson F, Kvist A, et al. Increased survival among HIV-1 and HIV-2 dual-infected individuals compared to HIV-1 single-infected individuals. AIDS. 2014;28:949-57.
28. Reeves JD, Hibbitts S, Simmons G, et al. Primary human immunodeficiency virus type 2 (HIV-2) isolates infect CD4-negative cells via CCR5 and CXCR4: comparison with HIV-1 and simian immunodeficiency virus and relevance to cell tropism in vivo. J Virol. 1999;73:7795-804.
29. Azevedo-Pereira JM, Santos-Costa Q, Taveira N, et al. Construction and characterization of CD4-independent infectious recombinant HIV-2 molecular clones. Virus Res. 2003;97:159-63.
30. Azevedo-Pereira JM, Santos-Costa Q, Mansinho K, et al. Identification and characterization of HIV-2 strains obtained from asymptomatic patients that do not use CCR5 or CXCR4 coreceptors. Virology. 2003;313:136-46.
31. Santos-Costa Q, Parreira R, Moniz-Pereira J, et al. Molecular characterization of the env gene of two CCR5/CXCR4-independent human immunodeficiency 2 primary isolates. J Med Virol. 2009;81:1869-81.
32. Santos-Costa Q, Mansinho K, Moniz-Pereira J, et al. Characterization of HIV-2 chimeric viruses unable to use CCR5 and CXCR4 coreceptors. Virus Res. 2009;142:41-50.
33. Moore JP. Simple methods for monitoring HIV-1 and HIV-2 gp120 binding to soluble CD4 by enzyme-linked immunosorbent assay: HIV-2 has a 25-fold lower affinity than HIV-1 for soluble CD4. AIDS. 1990;4:297-305.
34. Bahraoui E, Benjouad A, Guétard D, et al. Study of the interaction of HIV-1 and HIV-2 envelope glycoproteins with the CD4 receptor and role of N-glycans. AIDS Res Hum Retroviruses. 1992;8:565-73.
35. Morikawa Y, Moore JP, Wilkinson AJ, et al. Reduction in CD4 binding affinity associated with removal of a single glycosylation site in the external glycoprotein of HIV-2. Virology. 1991;180:853-6.
36. Endres MJ, Clapham PR, Marsh M, et al. CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. Cell. 1996;87:745-56.
37. Hoffman TL, LaBranche CC, Zhang W, et al. Stable exposure of the coreceptor-binding site in a CD4-independent HIV-1 envelope protein. Proc Natl Acad Sci USA. 1999;96:6359-64.
38. Kolchinsky P, Kiprilov E, Sodroski JG. Increased neutralization sensitivity of CD4-independent human immunodeficiency virus variants. J Virol. 2001;75:2041-50.
39. Yoshii H, Kamiyama H, Goto K, et al. CD4-independent human immunodeficiency virus infection involves participation of endocytosis and cathepsin B. PLoS One. 2011;6:e19352.
40. Yoshii H, Kamiyama H, Minematsu K, et al. Cathepsin L is required for ecotropic murine leukemia virus infection in NIH3T3 cells. Virology. 2009;394:227-34.
41. Kumar P, Nachagari D, Fields C, et al. Host cell cathepsins potentiate Moloney murine leukemia virus infection. J Virol. 2007;81:10506-14.
42. Chandran K, Sullivan NJ, Felbor U, et al. Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection. Science. 2005;308:1643-5.
43. Kubo Y, Hayashi H, Matsuyama T, et al. Retrovirus entry by endocytosis and cathepsin proteases. Adv Virol. 2012;2012:640894.
44. Connor RI, Sheridan KE, Ceradini D, et al. Change in coreceptor use correlates with disease progression in HIV-1--infected individuals. J Exp Med. 1997;185:621-8.
45. Tersmette M, Lange JM, de Goede RE, et al. Association between biological properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. Lancet. 1989;1:983-5.
46. Calado M, Matoso P, Santos-Costa Q, et al. Coreceptor usage by HIV-1 and HIV-2 primary isolates: the relevance of CCR8 chemokine receptor as an alternative coreceptor. Virology. 2010;408:174-82.
47. Berkowitz RD, Beckerman KP, Schall TJ, et al. CXCR4 and CCR5 expression delineates targets for HIV-1 disruption of T cell differentiation. J Immunol. 1998;161:3702-10.
48. Jekle A, Keppler OT, de Clercq E, et al. In vivo evolution of human immunodeficiency virus type 1 toward increased pathogenicity through CXCR4-mediated killing of uninfected CD4 T cells. J Virol. 2003;77:5846-54.
49. Salazar-Gonzalez JF, Salazar MG, Keele BF, et al. Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. J Exp Med. 2009;206:1273-89.
50. Jiang C, Parrish NF, Wilen CB, et al. Primary infection by a human immunodeficiency virus with atypical coreceptor tropism. J Virol. 2011; 85:10669-81.
51. Reeves JD, Doms RW. Human immunodeficiency virus type 2. J Gen Virol. 2002;83:1253-65.
52. Santos-Costa Q, Lopes MM, Lopes M, et al. HIV-2 interaction with cell coreceptors: amino acids within the V1/V2 region of viral envelope are determinant for CCR8, CCR5 and CXCR4 usage. Retrovirology. 2014; 11:99.
53. Wu Y, Yoder A. Chemokine coreceptor signaling in HIV-1 infection and pathogenesis. PLoS Pathog. 2009;5:e1000520.
54. Woods TC, Roberts BD, Butera ST, et al. Loss of inducible virus in CD45RA naive cells after human immunodeficiency virus-1 entry accounts for preferential viral replication in CD45RO memory cells. Blood. 1997;89:1635-41.
55. Xue B, Mizianty MJ, Kurgan L, et al. Protein intrinsic disorder as a flexible armor and a weapon of HIV-1. Cell Mol Life Sci. 2012;69:1211-59.
56. Tokuriki N, Oldfield CJ, Uversky VN, et al. Do viral proteins possess unique biophysical features? Trends Biochem Sci. 2009;34:53-9.
57. Liu J, Bartesaghi A, Borgnia MJ, et al. Molecular architecture of native HIV-1 gp120 trimers. Nature. 2008;455:109-13.
58. Choe H, Farzan M, Sun Y, et al. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell. 1996;85:1135-48.
59. Cocchi F, DeVico AL, Garzino-Demo A, et al. The V3 domain of the HIV-1 gp120 envelope glycoprotein is critical for chemokine-mediated blockade of infection. Nat Med. 1996;2:1244-7.
60. Svicher V, Alteri C, Artese A, et al. Identification and structural characterization of novel genetic elements in the HIV-1 V3 loop regulating coreceptor usage. Antivir Ther. 2011;16:1035-45.
61. Albert J, Stalhandske P, Marquina S, et al. Biological phenotype of HIV type 2 isolates correlates with V3 genotype. AIDS Res Hum Retroviruses. 1996;12:821-8.
62. Isaka Y, Sato A, Miki S, et al. Small amino acid changes in the V3 loop of human immunodeficiency virus type 2 determines the coreceptor usage for CXCR4 and CCR5. Virology. 1999;264:237-43.
63. Shi Y, Brandin E, Vincic E, et al. Evolution of human immunodeficiency virus type 2 coreceptor usage, autologous neutralization, envelope sequence and glycosylation. J Gen Virol. 2005;86:3385-96.
64. Visseaux B, Hurtado-Nedelec M, Charpentier C, et al. Molecular determinants of HIV-2 R5-X4 tropism in the V3 loop: Development of a new genotypic tool. J Infect Dis. 2012;205:111-20.
65. Owen SM, Ellenberger D, Rayfield M, et al. Genetically divergent strains of human immunodeficiency virus type 2 use multiple coreceptors for viral entry. J Virol. 1998;72:5425-32.
66. Kulkarni S, Tripathy S, Agnihotri K, et al. Indian primary HIV-2 isolates and relationship between V3 genotype, biological phenotype and coreceptor usage. Virology. 2005;337:68-75.
67. Lyumkis D, Julien J-P, de Val N, et al. Cryo-EM structure of a fully glycosylated soluble cleaved HIV-1 envelope trimer. Science. 2013;342: 1484-90.
68. Julien J-P, Cupo A, Sok D, et al. Crystal structure of a soluble cleaved HIV-1 envelope trimer. Science. 2013;342:1477-83.
69. Pinter A, Honnen WJ, He Y, et al. The V1/V2 domain of gp120 is a global regulator of the sensitivity of primary human immunodeficiency virus type 1 isolates to neutralization by antibodies commonly induced upon infection. J Virol. 2004;78:5205-15.
70. Rusert P, Krarup A, Magnus C, et al. Interaction of the gp120 V1V2 loop with a neighboring gp120 unit shields the HIV envelope trimer against cross-neutralizing antibodies. J Exp Med. 2011;208:1419-33.
71. Barroso H, Borrego P, Bártolo I, et al. Evolutionary and structural features of the C2, V3 and C3 envelope regions underlying the differences in HIV-1 and HIV-2 biology and infection. PLoS One. 2011;6:e14548.
72. McKnight A, Shotton C, Corbeil J, et al. Location, exposure, and conservation of neutralizing and nonneutralizing epitopes on human immunodeficiency virus type 2 SU glycoprotein. J Virol. 1996;70:4598-606.
73. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med. 2012;366:1275-86.
74. Hamoudi M, Simon-Loriere E, Gasser R, et al. Genetic diversity of the highly variable V1 region interferes with Human Immunodeficiency Virus type 1 envelope functionality. Retrovirology. 2013;10:114.
75. Marcelino JM, Borrego P, Nilsson C, et al. Resistance to antibody neutralization in HIV-2 infection occurs in late stage disease and is associated with X4 tropism. AIDS. 2012;26:2275-84.