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HIV-2 and the Immune Response

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Abstract

HIV-1 and HIV-2 infections have important differences in epidemiology, clinical progression and transmission. Studies of the less transmissible and pathogenic HIV-2 have revealed some intriguing facts, indicating that it is less prone to replicate and perhaps can evoke a more efficient or long-lasting immune response than HIV-1 in the human host. Several crucial aspects of HIV-2 infection are still insufficiently characterised. However, there is now convincing evidence that plasma viral load is considerably lower for HIV-2 than for HIV-1, despite similar proviral (DNA) loads for the two viruses. There are reports on lower levels of apoptosis for HIV-2, possibly indicating a lower level of harmful immune activation. Several studies have also shown that vigorous HIV-2 specific immune responses can be detected, especially during the asymptomatic phase of HIV-2 infection. This includes humoral as well as cell-mediated immunity (CMI). The neutralising antibody response appears to be broader and the CMI may be more efficient for HIV-2 as compared to HIV-1. However, comparative studies in the same population groups on HIV-1 and HIV-2 immunity are scarce and difficult to perform. Nevertheless, by increasing our knowledge about how HIV-2 is contained to a higher degree than HIV-1, clinically as well as epidemiologically, we may gain knowledge that is useful in a wider perspective in our struggle to curb the devastating HIV/AIDS epidemic.

Keywords

HIV-2. Immunology. Pathogenesis.

Introduction

While the human immunodeficiency virus type 1 (HIV-1) is on a devastating global trail causing immense suffering and death, the other human immunodeficiency virus, HIV-2, describes a much more limited path[1]. Although sharing significant properties, the two viruses exhibit some important differences in their biology and epidemiology[2,3] (Table 1). The reasons for these differences are largely unexplained and while the amount of knowledge about HIV-1 has grown at a tremendous pace, we still lack data on several crucial aspects of HIV-2. Learning more about HIV-2 may provide clues about how to cope with HIV-1. This review will focus on some new data relating to the interplay between HIV-2 and the host defence system, also including data from experimental non-human primate models. Other aspects including the epidemiology and biology of HIV-2 have been covered by previous reviews[2-4].

While HIV-1 has a global spread, HIV-2 is confined mainly to west Africa where the highest prevalence rates have been reported from Guinea-Bissau. Fairly high numbers of cases have also

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Table 1. *Comparison between HIV-1 and HIV-2.*

	HIV-1	HIV-2
Routes of transmission	No difference	
Geographical distribution	Global	West Africa (Portugal, India)
Age specific prevalence	Peak at 20-40 years	Increases with age
Vertical transmission*	15-40 %	< 5%
Heterosexual transmission	Significantly lower for HIV-2 than for HIV-1	
Time to AIDS*	±10 years	Significantly longer than for HIV-1
Proviral load (DNA)	No difference	
Plasma RNA level	Significantly lower for HIV-2 than for HIV-1	
Genetic comparison	40-60% homology	

** Without antiretroviral treatment.*

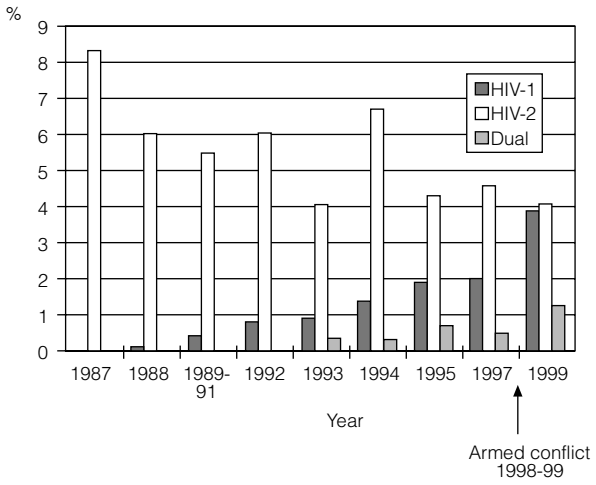

Fig. 1. *Seroprevalence of HIV-1, HIV-2 and HIV-1+2 dual infections among pregnant women in Guinea-Bissau, west Africa, 1987-99. Approximately 1,500 women were tested in each sample, apart from 1.987 (n = 707) and 1988 (n = 2539). The groups from 1989-91 were pooled due to small sample sizes (around 500 per year). From Ref. 14 and Z. da Silva & H. Norrgren, unpublished.*

appeared in Portugal and, more recently, in India. Occasional cases have been reported from many other parts of the world[2,5]. Several studies have shown that HIV-2 is associated with a lower transmission rate, vertical as well as sexual, and a reduced disease development rate compared to HIV-1[2-9].

The HIV-2 prevalence increases with age both among women and men[2,10,11]. Population based studies in Guinea-Bissau have shown peak prevalence rates around 60 years of age and it has been suggested that a cohort effect as a result of high transmission during the war of liberation in Guinea-Bissau some 30 years ago may be a reason for the higher prevalence rates in the older age groups[12,13]. More recent data indicate, though, that the HIV-2 prevalence in Guinea-Bissau now is declining, as observed among men in a community study[12] and also in pregnant women in Bissau[14] (Fig. 1). However, the HIV-2 prevalence appears to remain high among women in the older age groups[12]. A risk-factor analysis of wives of HIV-2 infected men reported age above 45 years as the major significant predictor of HIV-2 transmission[15]. Some support for this finding is provided by a study of published population-based surveys of human retrovirus infections in Africa showing increased female: male prevalence ratios in older age groups compared to younger age groups[16]. The reasons for the possible higher susceptibility of older women to HIV-2 transmission remain to be elucidated, but could include differences in sexual behaviour as well as immunological/hormonal factors.

HIV-1 and HIV-2 differ by 40-60% at the nucleotide level[17] and HIV-2 is more closely related to some of the SIVs than to HIV-1. Up to seven subtypes of HIV-2 have been recognised but only two, subtype A and B, appear to be of any epidemiological significance[18-20]. Subtype A is predominant throughout west Africa, although in Côte d'Ivoire a mixed epidemic of subtype A and B has been described[21-23]. The clinical significance of HIV-2 subtypes is still unclear. However, Simon *et al.* reported a higher cross-reactivity of HIV-2 subtype B with HIV-1 in some serological assays as compared to HIV-2 subtype A, giving rise to a larger number of HIV-1 and HIV-2 dually reactive cases based on serological diagnosis[24].

Switzer *et al.* reported data indicating a higher degree of *nef* truncations, as screened by a coupled transcription and translation assay for *nef* open reading frame interruptions, among HIV-2-infected

Table 2. *Cross-reactivity between HIV-1 and HIV-2 of serological assays with capacity to discriminate between the two viruses when testing 293 HIV-positive samples from Guinea-Bissau. Modified from ref. 31.*

Assay	Frequency of dual reactivity (%)
All assays	9.9
Western blot[a]	
any two env[b]	22.5
at least gp41/gp36[c]	14.7
Inno-LIA	25.2
Multispot	12.6
Pepti-LAV	10.9
Immunocomb Bispot	10.9
Wellcozyme HIV-1 ELISA[d]	
cut-off: absorbance ratio ≥ 1.0	23.6
cut-off: absorbance ratio ≥ 2.0	16.1

[a]Diagnostic Biotechnology HIV-blot 2.2 for HIV-1 and in-house assay for HIV-2.
[b]Requiring any two *env* bands, including gp120 and gp160, without gp41 of HIV-1.
[c]Requiring at least HIV-1 gp41 or HIV-2 gp36 plus any other *env* band of each WB.
[d]Cross-reactivity only assessed for HIV-2, 186 samples tested.

asymptomatic individuals as compared to HIV-1 infected patients at comparable clinical stages[25]. However, the role of *nef* in HIV-2 immunity and pathogenicity is still unclear.

Laboratory diagnostics

HIV-specific antibody production constitutes an important aspect of the immune response, providing the means for a majority of the routine diagnostic tools currently in use. In the early days of HIV diagnostics, after the discovery of HIV-2, cross-reactivity between HIV-1 and HIV-2 formed the basis for laboratory assays. The assays were usually composed of HIV-1 whole viral lysate antigen. When the differences between HIV-1 and HIV-2 started to become unravelled, it also became clear that it would be important to differentiate between the two viruses in the laboratory diagnostics. Early studies showed that the cross-reactivity was higher between antigens derived from the *gag* and *pol* regions of the viruses, while *env* derived proteins were able to differentiate to a higher degree[26,27]. The most common methodology for detection (screening) of HIV antibodies is the enzyme-linked immunosorbent assay (ELISA) and today there are excellent ELISA screening assays containing both HIV-1 and HIV-2 antigens. It is common routine to confirm antibody screening positive results with a second "confirmatory" assay. This is often a western blot (WB) assay, although some laboratories may use immunofluorescence assays (IFA) or, even less common at present, radio-immunoprecipitation assays (RIPA). Type specific WBs provide some degree of differentiation between HIV-1 and HIV-2, especially if the criteria elaborated by the World Health Organization are applied, requiring reactivity by at least two *env* bands[28]. More recently developed type-specific antibody assays designed to discriminate between HIV-1 and HIV-2 have improved the diagnostic procedure (Table 2). The inclusion of these assays in alternative-confirmatory strategies has today made it possible to screen and confirm based on a combination of ELISAs and/or rapid simple assays only[29-32], which is especially important in low-resource areas. By carefully choosing a proper combination of assays, a high degree of accuracy may be achieved including differentiation between HIV-1 and HIV-2 or confirmation of dual reactivities comparable to differentiation by PCR[31,33-35]. Some previous seroepidemiological data may have given incorrect prevalence rates of HIV-2 due to the use of diagnostic strategies not optimised for HIV-2 detection and confirmation. This may be exemplified by a recent report confirming a relatively high number of HIV-2 infected individuals in New York City, USA, discovered after the introduction of improved laboratory diagnostics for HIV-2[36].

PCR has also become a fairly common tool when antibody assays do not suffice, e.g. for diagnostics of early infections and in cases of indeterminate antibody test results.

Studies of viral load

The recent developments of various assays for determination of HIV-2 plasma RNA levels have led to a number of reports confirming the hypothesis and previous indirect data indicating that the differences in biology and epidemiology of HIV-1 and HIV-2 may be associated with differences in viral load[37,38]. Early studies of proviral DNA levels (in PBMCs) showed no difference between HIV-1 and HIV-2 infected individuals[21,39-41]. More recently, Berry *et al.* reported from a cross-sectional study of patients, stratified according to percentage $CD4^+$ T-lymphocytes (CD4%), that HIV-2-infected individuals had lower plasma RNA levels than HIV-1-infected persons at high CD4% levels, while at low CD4% levels the plasma RNA levels were similar[42]. No persons with known seroconversion dates were included in that study and the HIV-2 viral load measurements were performed by an in-house RT-PCR based on LTR primer sequences.

In a study among female sex workers (FSWs) in Senegal using an RT-PCR that amplified a portion of the *gag* region of HIV-2, the median viral load was 30 times lower in the HIV-2-infected compared to HIV-1-infected women and the plasma RNA levels were inversely related to CD4+ T-lymphocyte counts. The differences between HIV-1 and HIV-2 appeared to persist, irrespective of length of time infected[43].

Shanmugam *et al.* used an Amp-RT assay for comparative measurements of plasma RT activity as a marker for viral expression in a cross-sectional sample of HIV-1 and HIV-2-infected persons[44]. They, too, found a markedly lower level of plasma RT activity in HIV-2 as compared to HIV-1 infected individuals. In persons with CD4+ cell counts > 500 x 10^6/L, all HIV-2 carriers studied had undetectable RT-based plasma virus loads, as compared to 48% of the HIV-1 carriers. The differences were also evident among tuberculosis patients[44].

Through the development of a methodology for detection of plasma RNA levels for HIV-2, similar to a well-established plasma viral load assay for HIV-1 (by Roche), we could make direct comparisons of plasma viral load among known seroconverters (less than two years after seroconversion) and patients with symptoms, as well as in subjects with HIV-1 and HIV-2 dual infection. The results showed that HIV-2-infected persons appear to reach a significantly lower setpoint than HIV-1-infected individuals after seroconversion (28 times lower than HIV-1 in this study)[45]. There was a clear inverse correlation between the HIV-2 viral load and the CD4+ lymphocyte levels; the HIV-2 plasma viral load was approximately 1 log lower than HIV-1 throughout the comparable spectrum of CD4+ lymphocyte levels.

Moreover, it has been reported that the low rate of mother-to-child transmission of HIV-2 was associated with low maternal HIV-2 RNA levels (more than 30-fold less than HIV-1)[46].

Thus, reports are now accumulating from different study groups, using different methodologies, concordantly showing that HIV-2 plasma RNA levels are significantly lower than HIV-1, from early stages of the infection. The median plasma HIV-2 RNA levels detected in the various studies are comparable with those of many HIV-1-infected long-term non-progressors[47,48]. As demonstrated by Mellors *et al.*, the lower the viral load early after primary HIV-1 infection, the slower the rate of progression to AIDS[49]. If the data now obtained for HIV-2, including our group of seroconverters[45], are applied for the model presented by Mellors *et al.*, HIV-2 infection clearly resembles a slow or "non"-progressing HIV-1 infection[3,50]. It may thus be tempting to conclude that HIV-2 infection progresses more slowly than HIV-1 infection and is less transmissible, simply because plasma viral load is lower. However, the interplay between the virus and the host is complicated and further studies are clearly warranted in order to improve our understanding of these mechanisms. For instance, it has been shown that sooty mangabey monkeys may harbour high plasma concentrations of SIV, despite the lack of disease[51]. It appears that the lack of pathogenicity of SIVsm for its natural host cannot be explained by limited viral replication or by tight containment of viral production. The mechanisms through which HIV-2 infection in humans is contained to a higher degree than HIV-1 remain to be identified. Differences in the interaction between the human immune system and the two HIV types are a tempting explanation. Inherent differences in the viral biology is yet another possibility.

CD4+ lymphocyte decline and apoptosis

The rate of decline of CD4+ lymphocytes is considerably slower in HIV-2-infected persons compared to HIV-1 carriers. Yet, some individuals infected with HIV-2 may suffer a rapid progression to AIDS[40]. When reaching the later stages of HIV-2 infection, there is an impairment of CD4+ lymphocyte function similar to what is seen in HIV-1-infected patients[2,4,5,52,53].

Michel and co-workers recently reported that immune activation measured as expression of the activation marker HLA-DR on T lymphocytes was lower in HIV-2 than in HIV-1-infected persons. Possibly as a consequence, the *ex vivo* apoptosis was lower in HIV-2 than in HIV-1 infection and there was a high correlation between the level of CD4+ T-cell apoptosis and serum β2-microglobulin concentration and disease progression[54]. These findings are in line with the only previously published study on lymphocyte cell death and apoptosis in HIV-2 infection, where a significantly lower level of in vitro T-cell apoptosis was found among asymptomatic HIV-2 carriers compared to HIV-1-infected individuals at comparable disease stages. The HIV-2 group was similar to healthy HIV-negative controls[55]. Moreover, Cavaleiro *et al.* showed that gp105 of HIV-2_{ROD} had an inhibitory effect on T cell proliferation and the up-regulation of CD40L and OX40, which are co-stimulatory molecules important in the activation and differentiation of the T-cell response (as well as dendritic cell maturation). This immunosuppressive effect was accompanied by a reduced level of apoptosis[56].

Taken together, it may be speculated that a weaker long-term activation of the immune system in HIV-2 infection compared to HIV-1 contributes to the slower T-cell depletion and disease evolution. The importance of a general, non-HIV-specific, immune activation for viral replication and disease progression in HIV infection has previously been pointed out[57-60]. However, the possible mechanisms behind the different levels of immune activation for HIV-1 and HIV-2 are poorly understood.

Antibody neutralization of HIV-2

IgM and IgG antibodies against the structural proteins of HIV develop early after primary infection and the latter usually remain throughout the course of infection[61,62]. The antibody response against the *gag* protein is often undetectable during the late stages of the disease, while the antibodies to the envelope proteins usually are maintained. Antibodies with a neutralising effect on live virus particles (neutralising antibodies; NA) seem to constitute a

much smaller fraction of the total anti-HIV antibodies developed during HIV infection than the "diagnostic" antibodies[61,62].

Data from several studies indicate that NA play a role in preventing or modulating infection with HIV, SIV or SHIV[62-64]. Broader and higher frequencies of autologous NA have been demonstrated in HIV-1-infected long-term non-progressors (LTNP) and slow progressors, compared with other HIV-1-infected individuals. Sera from mothers who did not transmit virus to their children had a capacity to cross-neutralise several HIV-1 isolates, whereas sera from HIV-transmitting mothers did not have this capacity[65]. Hence, it is logical that autologous neutralising antibodies have been found more frequently in HIV-2-infected than in HIV-1-infected individuals[66] and it has been suggested that this difference in virus-neutralising activity may contribute to the slower disease progression in HIV-2 infection.

Cross-neutralisation between HIV-1 and HIV-2 have been shown in several studies, albeit with some conflicting results. Weiss *et al.* showed that human HIV-2 antisera could cross-neutralise HIV-1, whereas HIV-1 sera were type-specific[67]. Böttiger *et al.* showed bi-directional cross-neutralisation[68,69] while Robert-Guroff *et al.* demonstrated weak cross-neutralisation between HIV-1 and HIV-2[70]. While all these studies used continuous cell-lines in their assays, a study by Nyambi *et al.* on human PBMCs showed weak cross-neutralisation between HIV-1 and HIV-2; being more extensive between HIV-1 and SIVcpz[71]. It was reported from immunisation experiments in rabbits that peptide antisera directed against HIV-2 reverse transcriptase and integrase also cross-reacted with corresponding HIV-1 proteins, although HIV-1 sera were type specific[72]. HIV-2 infected women had a more pronounced cross-reactivity of cervicovaginal anti-HIV-2 IgG and IgA antibodies to HIV-1 epitopes than conversely[73].

Antibody patterns have also, similar to what can be observed in the course of HIV-1 infection, been suggested to have an association with disease development; the absence of anti-p26 based on immunoblot in early asymptomatic stages was in one study a predictor of more rapid disease progression, especially in combination with the occurrence of anti-vpx[74].

It is well established that the third variable region V3 of the envelope glycoprotein contains neutralising sites for both HIV-1 and HIV-2. More specifically, Björling *et al.* showed, by the use of synthetic peptides blocking NAs, that two linear sites of the HIV-2 V3 region, amino acids 312-315 and 329-331, were important for binding[75]. Two other groups have shown that monoclonal antibodies directed to the same region could exert an effective strain or isolate specific neutralising activity[76,77]. More recently, Skott and Mörner and co-workers have characterised in guinea-pig experiments three antigenic determinants located in the V2 and V4 regions of the HIV-2 *env* gp125, and one region of gp36, which appear to be of importance for antibody binding and as targets for neutralisation[78,79].

The study of antibody responses in HIV-2 exposed non-infected individuals (FSWs in The Gambia) could not demonstrate HIV-specific vaginal IgA or IgG, nor did the vaginal secretions display any HIV-neutralising activity[80].

Antibodies can also be active in antibody-dependent cellular cytotoxicity (ADCC). The role of ADCC in protection against HIV infection or AIDS development is not completely clear[81-83]. In chimpanzees, which do not usually develop disease after HIV-1 infection, a delayed ADCC or lack thereof has been reported, while in humans a strong ADCC response is often demonstrable[63]. There have been speculations that ADCC may be harmful, e.g. eliminating non-infected cells that have adsorbed viral env-components to their surface[63]. However, Connick *et al.* have shown that ADCC may be important for the control of viral replication in acute HIV-1 infection[84]. In addition, Baum *et al.* described high levels of ADCC-mediating antibodies in LTNP, while rapid progressors had significantly lower titres[85]. HIV-2 induces ADCC in a majority of infected individuals. The response appears to be of broader specificity and higher frequency than what has been shown for HIV-1[83,86-88]. ADCC has also been demonstrated in HIV-2-immunised as well as infected monkeys. In the immunized, non-infected monkeys, the ADCC response diminished over time and required booster immunisations to persist[89].

Data on NA and ADCC in relation to clinical progression or non-progression and to exposure to HIV-2 are incomplete. While it is generally easier to demonstrate neutralising activity that is strain or isolate-specific, it is now important to identify epitopes against which NAs can mediate broad cross-clade neutralising activity involving wild-type viruses. The general picture of broader specificities and cross-reactivities of anti-HIV-2 sera could provide clues to vaccine-design experiments.

Cell-mediated immune responses

There is mounting evidence that cell-mediated immune responses are important for protection against and control of HIV/AIDS[90,91]. Several groups have provided evidence for HIV-1-specific CTL and T-helper cell responses in individuals who are able to cope with exposure to HIV without becoming infected, as well as in HIV-1-infected LTNP[92-95]. Rosenberg *et al.* showed that HIV-1-infected LTNP and persons with primary HIV-1 infection who had received early antiviral therapy had high T-helper cell responses to *gag* and *env* antigens. They also showed an inverse relationship between plasma HIV-1 viral load and the magnitude of the T-helper responses[96]. These findings contrast with previous reports of low-grade T-helper responses in individuals with chronic HIV-1 infection[97-99]. Taken together, these observations could indicate that a chronic, progressive HIV infection will develop if the HIV-specific cell-mediated immune response is not sufficient enough to contain the infection[100].

Data on specific cell-mediated immune responses to HIV-2 are scarcer. Pinto *et al.* demonstrated the

occurrence of T-helper cell responses to synthetic SIV and recombinant HIV-2 antigens in HIV-2-infected individuals[101,102]. CTL activity has been demonstrated in a majority (7 of 9 studied) of asymptomatic HIV-2 carriers, directly from peripheral blood[103] and in re-stimulated effector cells from PBMCs (15 of 18 studied)[104]. Two of six highly exposed FSWs in Gambia were shown to have both HIV-1 and HIV-2-specific CTL[105]. Ariyoshi *et al.* reported an inverse correlation between HIV-2-specific CTL activity and HIV-2 proviral load in 20 HIV-2-infected individuals studied[106]. HIV-2 *gag*-specific CTL were shown to frequently (9 of 11 studied) cross-react with HIV-1 *gag* expressed in vaccinia virus recombinant infected target cells[104]. Some CTL epitopes mediate cross-reactivity between HIV-1 and HIV-2 while others do not, despite close relationship[105,107,108]. In Guinea-Bissau we have demonstrated the occurrence of an anti-HIV-2 specific T-helper response in nearly half of the HIV-2 - infected individuals tested. Furthermore, increased anti-HIV-2 specific T-cell proliferative responses were also found in HIV-2-exposed, but non-infected persons as compared to HIV-seronegative presumed non-exposed controls[109].

The non-cytolytic soluble $CD8^+$ T-cell antiviral factor (CAF)[110], has not been studied in human HIV-2 infection as yet; only in macaque and baboon HIV-2 experiments (below). Little is also known about cytokine profiles in HIV-2 infection. However, Sekigawa and co-workers reported that recombinant HIV-2 *env* glycoprotein could stimulate a higher production of INF-γ and IL-16 than HIV-1 *env* could[111]. Both INF-γ and IL-16 can inhibit viral replication.

In summary, HIV-2 specific cell-mediated immune responses seem to prevail in a larger proportion of HIV-2 carriers than among HIV-1-infected persons. This resembles the situation in HIV-1-exposed non-infected or LTNP groups and suggests that a more vigorous and effective immunity is mounted in response to HIV-2 which might lead to a lower rate of virus replication. However, the number of HIV-2-infected individuals studied is still limited and further studies are required to reach more convincing conclusions.

Chemokines and coreceptors

Chemokines are small cytokine-like soluble proteins, which act in the process of chemotaxis of leukocytes. Chemokines appear early in the immune response and one of their main duties is to recruit immune-competent cells to the action site. In any virus infection, presumably also HIV, a rapid chemokine response may occur early in infection while a specific immune response is being built up[112,113].

Several chemokine receptors can be utilised by HIV for cell entry, usually in combination with the CD4 molecule. CCR5 and CXCR4 are the two main co-receptors for HIV-1, corresponding to the two main groups of chemokines, the α- and the β-chemokines, also named CC- and CXC-chemokines, respectively[114]. The chemokines may achieve their antiviral effect through direct blocking of the receptors, but it has also been shown that high chemokine concentrations may lead to down-regulation of the receptors expressed on the cell surface[115,116]. Recently the β-chemokines MIP-1a, MIP-1b, and RANTES were also shown to exert an anti-apoptotic effect on lymphocytes from HIV-infected as well as healthy non-infected individuals[117].

Data on chemokines in relation to HIV-2 infection are scarce. HIV-2 can use CCR5 and CXCR4 as co-receptor, but unlike HIV-1, also uses a whole range of other co-receptors for entry of target cells[118-122]. Certain laboratory-adapted HIV-2 strains have been shown to infect CD4-negative cells primarily through the CXCR4 receptor[123,124]. More recently, primary HIV-2 isolates were shown to infect CD4-negative cells via CXCR4 as well as CCR5[125]. Furthermore, the second extra-cellular loop of human CXCR4 was shown to be critical for this CD4-independent entry into target cells[126].

Schramm *et al.* investigated the impact of co-receptor usage on the cytopathicity of HIV-2 and found that HIV-2 co-receptor specificity for CCR5 or CXCR4 determined the target cell population for T-cell depletion in lymphoid tissues. CXCR4-using HIV-2 variants were found to be more cytopathic and comparable to that of HIV-1. These findings indicate that the direct cytopathic capacity by itself does not explain the lower pathogenicity and transmissibility of HIV-2 than HIV-1[127]. Van der Ende *et al.* reported from an *in vivo* human-to-mouse chimeric model, data indicating that broadening of the HIV-2 co-receptor usage and, thus, the potential cellular host-range does not necessarily lead to a higher pathogenicity[128]. Circumstantial evidence for that is already provided by the fact that HIV-2 co-receptor usage is more promiscuous than HIV-1 and yet the latter is significantly more pathogenic.

Kaneko and Akimoto *et al.* demonstrated that the HIV-2 *env* glycoprotein, in contrast to HIV-1 *env*, could bind to the α-chain of CD8 molecules on T cells[129,130]. This binding was shown to induce phosphorylation of protein tyrosine kinase $p56^{lck}$ in $CD8^+$ cells. They also demonstrated a higher b-chemokine production after HIV-2 *env* stimulation as compared to HIV-1 *env*[130]. The main source of b-chemokines was the $CD8^+$ cells. Despite the binding of HIV-2 *env* to CD8, the cells did not become infected by the virus[131]. The authors postulate that the binding of HIV-2 *env* leads to a signal transduction into $CD8^+$ cells and the following b-chemokine production. This could be one mechanism by which HIV-2 is rendered less prone to replicate than HIV-1. In a preliminary study we showed that HIV-2 exposed, but uninfected, individuals as well as HIV-2 infected patients had increased b-chemokine and INF-γ production by mitogen-stimulated $CD8^+$ T-cell-enriched cultures as compared to two healthy control groups consisting of adolescents from Bissau and Swedish blood donors[109]. The study was unfortunately interrupted by the recent war in Guinea-Bissau.

To our knowledge, no investigation of β-chemokines in relation to various clinical stages of HIV-2 infection in humans has been presented. Studies in non-human primate models provide further support for a role of β-chemokines in the protection against HIV-2 (below). Hence, it seems likely that β-chemokines play a role in the defence against HIV-2, but formal

proofs in well-controlled human studies are still insufficient. Several issues need further experimental verification, including the role of co-receptor usage in relation to pathogenicity and immunity, as reviewed by Edinger *et al.*[132].

Animal models

Monkey models have been useful for studies of immune responses to HIV-2. HIV-2 can replicate in macaques, although only some strains cause symptomatic disease. In contrast, an optimal animal model for HIV-1 has been difficult to establish. However, SIV and some chimeric SIV/HIV viruses (SHIVs, which are made up of an SIV genetic backbone expressing the HIV-1 *env* gene) are pathogenic for macaques, also allowing studies of disease progression[133].

Protective immunity against HIV-2 and SIV infections in monkeys has been induced by different immunogens[134-136], but it has been difficult to establish clear correlations between HIV/SIV specific immune responses and protection against infectious-challenge virus. However, accumulating evidence clearly indicate that both cytotoxic T-cell (CTL) and neutralising antibodies (NA) play a role, backed up by a strong T-helper response[4,91,137,138]. As mentioned above, these data have also found support in studies on humans, particularly regarding HIV-1 but in few studies for HIV-2.

In one of the few pathogenic HIV-2 monkey vaccine models it was demonstrated that Macaca nemestrina monkeys, immunised with an apathogenic HIV-2 molecular clone (HIV-2KR), were protected from CD4+ cell decline and disease upon challenge with a pathogenic HIV-2 variant (HIV-2287). Protection was dose-dependent and protected animals displayed substantial reductions in PBMC proviral burden (1-3 logs), viral titres (1-2 logs), and plasma viral RNA (2-4 logs) compared to unprotected or naïve animals. No neutralising responses could be demonstrated, but CTL activity was detected early and at higher levels after challenge in protected macaques[139].

In baboons, HIV-2 infection leads to persistent viraemia and, in some cases, AIDS-like clinical symptoms[140]. CD8+ lymphocytes from HIV-2-infected baboons were shown to develop anti-HIV-2 activity in vitro, mediated by a combination of a soluble antiviral factor and ability to kill virus-infected CD4+ lymphocytes[141]. Super-infection experiments of HIV-2-infected baboons with heterologous HIV-2 resulted in reactivation of the originally inoculated HIV-2 while the second virus was blocked. This resistance to super-infection was reportedly mediated by CD8+ cells and at least partially by the T-cell antiviral factor (CAF), the puzzling hitherto not completely characterised soluble factor produced by CD8+ cells, initially described by Levy and colleagues[142,143]. Along the same lines, inoculation of pig-tailed macaques (Macaca nemestrina) with two different HIV-2 subtype A isolates (GB122 and CDC77618) showed that dual infection could be established mainly during the first two weeks of infection with the first virus. Dual infection could not be established more than four weeks after the first infection; immune correlates were assessed in that study[144].

Infection of macaques with non-pathogenic HIV-2, acting as a live attenuated vaccine has been shown to elicit cross-protection against SIV-induced disease but not complete protection against SIV infection[145]. HIV-2 exposed non-infected monkeys were shown to harbour SIV-specific CTLs and were resistant to mucosal SIV challenge[146]. Moreover, a recent study in our laboratory has shown that these protected monkeys had increased production of β-chemokines and CAF-like activity in mitogen-stimulated $CD8^+$ T-cell enriched cultures, as compared to naïve controls[147]. These data are in line with findings by several other groups in SIV animal vaccine models[148-152]. Furthermore, our group has previously shown high production of chemokines in protected macaques even prior to vaccination[152]. Others have also reported that $CD8^+$ T-cell-mediated anti-viral activity of varying magnitude can be mounted in cells from naïve animals unrelated to immunisation[153].

Patterson *et al.* reported the somewhat more surprising finding that recombinant HIV-1 pox virus-immunised macaques were protected against subsequent challenge with $HIV\text{-}2_{SBL\text{-}6669}$, but not against SHIV challenge after vaccination with recombinant HIV-2 pox virus. This HIV-1-mediated HIV-2 cross-protection has now been achieved in two different experiments[154,155] and was accompanied by cross-reactive CTL and secretion of CAF, but not neutralising antibodies (NA), in some animals.

In the SIV_{sm}-macaque model, the specificity and titre of the NA response have been found to closely correlate with disease progression[156]. Passive immunisation studies in chimpanzees and macaques[64,157] have shown that monoclonal antibodies, serum or immunoglobulins from vaccinated or infected animals can protect against HIV-1, HIV-2[158], SHIV or SIV infection, or delay progression of SIV-induced disease. However, in some studies no protection against SIV was observed in passively immunised macaques. Studies of the role of NA in protective immunity in vaccinated primates have given contradictory results[64]. Correlation of NA with protection against infection in vaccinated animals has been demonstrated in the chimpanzee/HIV-1 model and the macaque/SHIV model (in HIV-1 vaccinated animals) but not in the HIV-2/macaque model[158] and rarely in the SIV/macaque model.

The animal model experiments have allowed the development of techniques for assessment of various immune responses in the process of immunisation and infection. Our group has established assays for cell-mediated immune responses as well as antibody tests including neutralising antibody assays[159]. Some of these techniques have then been adapted for human studies (see below). A general problem with non-human primate studies is the relatively limited number of animals that has been used in each experiment. Design of experiments so that they are comparable between different groups and/or pooling of results in a meta-analytic fashion can overcome some of these drawbacks and allow more extensive conclusions.

Table 3. *Studies of potential protective effect of HIV-2 against subsequent HIV-1 infection.*

	Study site (ref.)			
	Dakar[160]	**Abidjan[162]**	**Bissau[14]**	**Bissau[2]**
Population group	FSW	Pregnant women	Police officers (90% male)	Community study
No. HIV negative at study start	398	266	1511	729
No. HIV-2 positive at study start	199	127	185	
Incident HIV-1 cases/total no PYO:				
in previously HIV negative	44/2020	5/467	35/4704	7/3751
in previously HIV-2 positive	7/780	6/208	7/574	7/388
Incidence per 100 PYO (95% CI):				
negative to HIV-1	2.2 (1.6-2.9)	1.1 (0.3-2.5)	0.74 (0.5-1.0)	0.2 (0.05-0.3)
HIV-2 to dual	0.9 (0.4-1.9)	2.9 (1.1-6.3)	1.22	1.9 (0.4-3.3)
Incidence rate ratio	0.3 (0.09-0.7)	2.7 (0.7-11.2)	1.6 (0.7-3.7)	10.1 (3.5-28.9)
Method of diagnosing dual infection	PCR	Antibody detection	Antibody detection and PCR[c]	Antibody detection and PCR[c]

[a]Adjusted for age, nationality, years of registered prostitution, gonorrhoea infection status, calendar year.
[b]Preliminary report in ref 2.
[c]Diagnostic strategy for antibody detection highly concordant with type-specific PCR[31,35]
FSW, Female sex workers. STD, sexually transmitted diseases. CI, confidence interval. PYO, person years of observation. PCR, polymerase chain reaction.
Table updated from Ref 2 and 161.

Possible protective effect of HIV-2 against subsequent HIV-1 infection

The possibility of HIV-2 acting as a live-attenuated vaccine against HIV-1 is an attractive concept and a study in Senegal provided data in favour of this idea. A group of FSWs in Dakar was followed and it was shown that HIV-2 infection conferred a 52-74% protection against subsequent HIV-1 infection1[60,161] (Table 3). To some disappointment, subsequent studies in other areas of west Africa have not demonstrated the protective effect[14,162-164] (Table 3). On the contrary, these studies have shown trends towards increased incidence of HIV-1 among HIV-2-infected persons as compared to HIV-negative, although not with statistical significance. There may be several explanations for these discrepant results but one is the obvious difference in the study populations (FSWs in Senegal, pregnant women in Côte d'Ivoire and police officers, 90% male, in Guinea-Bissau).

However, as outlined above, in vitro studies have provided some support for the concept of interference between the two viruses, or one virus providing a cross-reactive response against the other. This includes earlier data of HIV-2 NA cross-reacting against HIV-1 but not the other way around[67-70,72,73] as well as cross-reactive CTL[104,105,107,108]. Another possibility could be b-chemokine production induced either as an effect of an immune response to HIV-2 or as a direct effect by HIV-2 *env* on CD8+ lymphocytes[129,130,165]. Inhibition or down-regulation of HIV-1 replication by HIV-2 by other mechanisms at the cellular level has also been suggested[166-169]. In any case, further proofs from population-based studies of the concept of natural immunisation by HIV-2 against HIV-1 are highly desired.

HIV-1 and HIV-2 dual infection

Despite the possibility of HIV-2 mediating protection against HIV-1, the increasing spread of the latter in previously HIV-2 endemic areas[14,37] has led to a growing number of HIV-1 and HIV-2 dually infected individuals. Guinea-Bissau constitutes a recent example of a country with a previously virtually exclusive HIV-2 endemic situation, with the highest prevalence rate of HIV-2 in the world, where HIV-1 now has entered, giving rise to a concomitant epidemic of the two types of HIV[14] (Fig. 1).

The few reports that exist about the clinical and immunopathological progression of dual infection indicate a different picture than HIV-2 single infection, more resembling HIV-1 infection, as assessed by the occurrence of AIDS-associated symptoms, CD4+/CD8$^+$ lymphocyte levels, serum immunoglobulin concentrations and levels of HLA-DR in CD4$^+$ and CD8$^+$ T cells[170-172]. In our own study cohorts in Guinea-Bissau there appear to be an overrepresentation of dually infected cases at late clinical stages (unpublished). However, this anecdotal information is in need of formal proofs.

In a study of dually-infected individuals in Guinea-Bissau there was no clear correlation neither between plasma viral load and CD4$^+$ lymphocyte levels nor between the HIV-1 and HIV-2 plasma RNA concentrations. The HIV-1 RNA load was significantly lower in dually rather than in singly infected individuals[45]. Nkengasong *et al.* compared plasma HIV-1 RNA concentrations in HIV-1 singly and HIV-1 and HIV-2 dually-infected FSWs at similar clinical stages and found no differences[172]. Possible reasons for the divergent findings in these two studies include differences in study populations, limited study group[45] and unknown history of infections, i.e. which virus infected the person first and

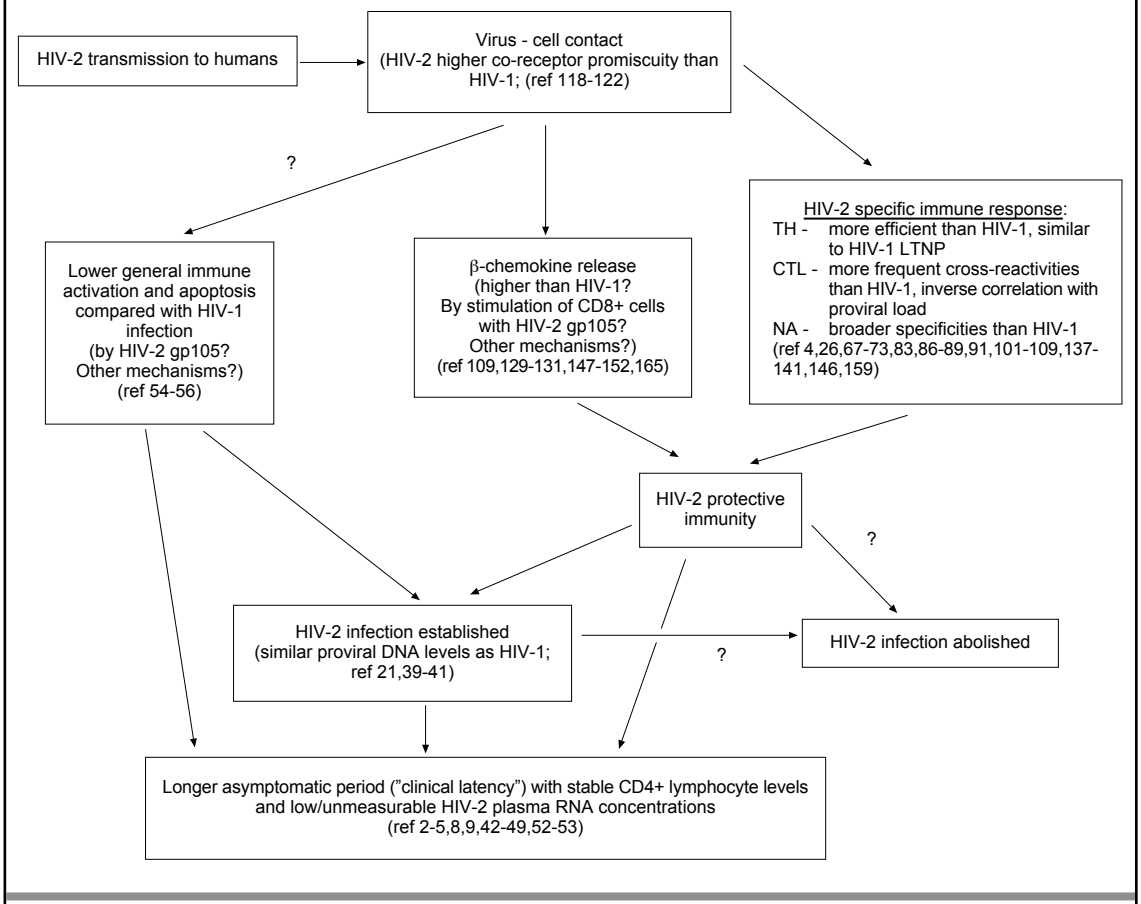

Fig. 2. *Model of possible mechanisms involved in the reduced pathogenicity of HIV-2 as compared to HIV-1*

length of time since seroconversion for each virus.

Dieng Sarr and co-workers reported the somewhat counter-intuitive observation that low HIV-2 proviral (DNA) load in dually-infected individuals correlated with low $CD4^+$ T-lymphocyte counts. For HIV-2 singly infected there was an inverse relationship of proviral load and $CD4^+$ T-cell values[173]. Possible explanations given by the authors for this finding include overgrowth of highly replicative HIV-1 strains at the expense of HIV-2, hiding of HIV-2 in other tissue reservoirs than PBMCs and, again, the possible influence of the order of infection. The study did not include determinations of plasma RNA or HIV-1 proviral load.

One study of super-infection with HIV-1 of HIV-2 infected cells resulted in phenotypically mixed virus particles with an expanded cellular host range, raising the possibility of a more rapid disease progression *in vivo*[174].

Thus, once dual infection has been established it may not be of any advantage as compared to carrying just one of the viruses. However, dual infection provides a tool for studies of the interactions between two distinct, albeit related, retroviruses in the same host. The mechanisms involved are complex and further studies clearly warranted.

Conclusions

Amid this catastrophic situation with an epidemic of unprecedented magnitude caused by a relatively recently described virus infection, nature is providing an important experiment for us[175]. The two known human lentiviruses HIV-1 and HIV-2 perform markedly differently in relation to their host, causing a pattern of transmission and clinical progression which is so divergent that comparative studies are likely to hold important clues as to how we should deal with these viruses in order to curb the HIV/AIDS epidemic. While we know a fair amount about the biological properties of HIV-1 and its interactions with the host, our knowledge of the inner secrets of HIV-2 is much more limited.

The recent new data on virological and biological properties of HIV-2 has lent support to the clinical and epidemiological picture of a much less pathogenic and transmissible virus. It appears that a lower level of general immune activation, possibly in combination with an efficient HIV-2 specific immune response, leads to a lower virus production in HIV-2 infection, despite similar levels of DNA template as for HIV-1 (Fig. 2). These mechanisms, specific as well as non-specific, need to be characterised in more detail.

Thus, further studies of HIV-2 are of utmost importance and several issues regarding the interplay between HIV-2 and the host defence system need to be addressed in future studies; these include studies of larger patient or animal groups, especially in experimental laboratory work, in different settings, focusing on some of the particularities of HIV-2, e.g. the higher incidence among older women, the role of chemokines and the possibility of a broader and

stronger immune response as compared to HIV-1. When possible, HIV-1 and HIV-2 should be compared in the same study populations, recognising that parallel studies of the effectiveness of the immune response against HIV-2 compared with HIV-1 are difficult to perform. Finally, the role of the innate immune system has not been studied in HIV-2 infection and should be a focus of future investigations.

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