

Pathogenesis of simian immunodeficiency virus infection

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Introduction

Simian immunodeficiency virus (SIV) comprises a family of lentiviruses that infect non-human primates. These viruses share important biological and genetic properties with human immunodeficiency virus (HIV), and SIV strains from sooty mangabey and macaque monkeys (SIV_{mac/smm}) have been widely employed in HIV/acquired immunodeficiency syndrome (AIDS) research, due to their ability to cause a range of clinical sequelae in macaques that approximate those seen in humans with AIDS. This review will provide a general overview of SIV infection and a discussion of SIV pathogenesis as it relates to the goal of understanding the disease processes that lead to AIDS; particular emphasis will be placed on SIV_{mac/smm} since this represents the most widely used experimental animal model for the pathogenesis of human AIDS.

General features of infection

Primate lentivirus families

Primate lentiviruses identified so far fall into five distinct groups, based on sequence relatedness: SIV_{smm}/SIV_{mac}/HIV-2, SIV_{mnd}/SIV_{l'hoest}, SIV_{syk}, SIV_{agm} and HIV-1/SIV_{cpz} (Fig. 1). In most cases, it is believed that the viruses have become highly adapted to their host species over an extended period of time – resulting in the ability to establish productive but non-pathogenic infections. The induction of immunodeficiency disease is typically a hallmark of infection of a new host species, to which the virus has not adapted itself (Gao *et al.*, 1999).

Apathogenic natural infections

A high incidence of natural SIV infection occurs in wild populations of African green monkeys (*Cercopithecus aethiops*),

and natural SIV infection has also been identified in wild sooty mangabey monkeys (*Cercocebus atys*) and mandrills (*Papio sphinx*). Macaques of Asian origin do not appear to be infected in the wild, although they may acquire SIV infection in

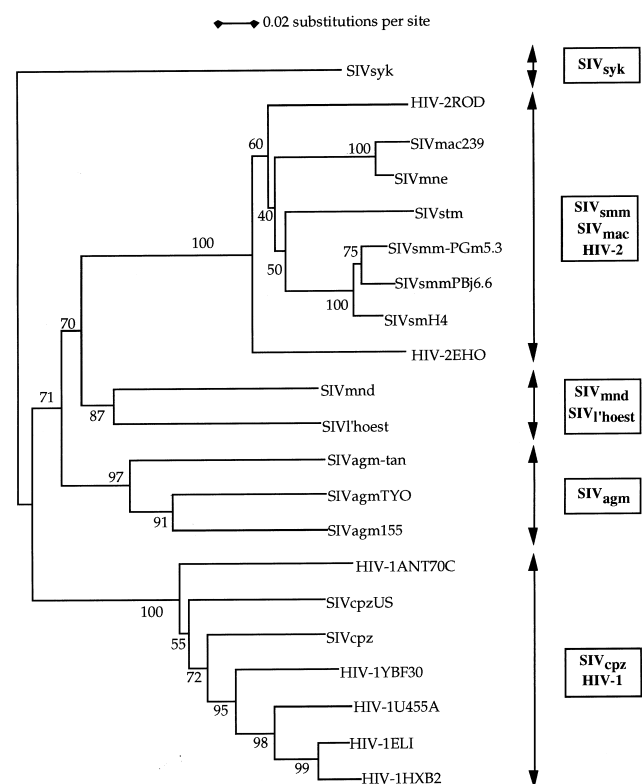


Fig. 1. Phylogenetic relationships of selected SIV and HIV *pol* sequences. The sequences were analysed using the neighbour-joining method of the ClustalX program. Horizontal branch lengths are to scale; vertical separation is for clarity. The numbers at each node indicate the percentage of bootstrap samples (out of 5000) in which the cluster to the right is supported. All sequences were obtained from GenBank. HIV-1 and HIV-2 strain names are essentially arbitrary, while SIV strain names include a prefix which identifies the species from which the viral sequences were cloned. Such prefixes include: *stm*, stump-tailed macaque; *smm/sm*, sooty mangabey; *mne*, pig-tailed macaque; *mac*, rhesus macaque; *mnd*, mandrill; *l'hoest*, l'hoest monkey; *syk*, Sykes monkey; *agm*, African green monkey; *tan*, tanzania monkey; *cpz*, chimpanzee. The boxes containing boldface type denote the five major families of primate lentiviruses which have been identified to date.

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captivity. Transmission in the wild occurs primarily through sexual activity and through male–male aggression (Jolly *et al.*, 1996; Nerrienet *et al.*, 1998; Phillips-Conroy *et al.*, 1994).

SIV does not cause illness in natural infections, despite continuous, high-level virus replication (Fultz *et al.*, 1990; Kurth & Norley, 1996; Rey-Cuille *et al.*, 1998). However, disease can result when the virus is transferred to a new host species, with a correlation between severity of disease and level of virus replication (Hirsch *et al.*, 1995; Lowenstine *et al.*, 1986; Murphey-Corb *et al.*, 1986; Ohta *et al.*, 1988). Several studies have sought to define parameters associated with apathogenic infections in natural hosts in order to understand better pathogenic infections of new host species, such as humans. To this end, investigators have focused particular attention on host responses to infection and on the rate and nature of virus genetic evolution.

A lower rate of virus evolution was found in rhesus macaques infected with SIV_{smm9} than in sooty mangabeys. This suggested that the natural host (mangabeys) might exert only a very modest selective pressure on the virus (Cournaud *et al.*, 1998). In contrast, broader and more sustained cytotoxic T lymphocyte (CTL) activity was detected in mangabeys infected with SIV_{mac239}, as compared to rhesus macaques (Kaur *et al.*, 1998). These apparently conflicting results highlight the difficulty inherent to studies of this kind, particularly when working with virus strains which have been selected for their virulence in ‘unnatural’ hosts such as the rhesus macaque.

Other differences in host immune responses to infection have also been detected in infections of natural and new host species. Two groups have noted the absence of virus trapping on follicular dendritic cells in the lymph nodes of naturally infected animals (Beer *et al.*, 1996; Rey-Cuille *et al.*, 1998), suggesting that the trapping of immune complexes on the surface of dendritic cells may contribute to destruction of lymph node architecture in pathogenic infections (Kurth & Norley, 1996). In conjunction with this, unusually low levels of antibody to Gag, which could participate in immune complex formation, were found in SIV_{agm}-infected African green monkeys (Norley *et al.*, 1990).

Infections of the neonate

As is the case for adults, infection of neonates with host-adapted variants is apathogenic (Beer *et al.*, 1998), while accelerated disease progression may occur following cross-species virus transmission (Baba *et al.*, 1994; Bohm *et al.*, 1993; Marthas *et al.*, 1995). In macaques, vertical transmission of SIV is relatively uncommon, and occurs primarily from ingesting virus-infected milk, as protective levels of maternal antibody wane (Otsyula *et al.*, 1995; Phillips-Conroy *et al.*, 1994). This raises intriguing questions concerning the route of virus entry following oral exposure to infectious virus. Recent studies by O’Neil and colleagues suggest that orally delivered virus may in fact be capable of traversing the stomach, and of infecting

lymphoid cells in the gastrointestinal mucosa (S. O’Neil, personal communication).

Transmission of natural and experimental infection

Experimental infection with SIV has been accomplished following oral, mucosal, intravenous and transplacental administration of virus (Baba *et al.*, 1995; Neildez *et al.*, 1998; Pauza *et al.*, 1998; Ruprecht *et al.*, 1998). The efficiency of vaginal transmission increases during the luteal phase of the menstrual cycle (Sodora *et al.*, 1998) and in animals treated with progesterone (Marx *et al.*, 1996). Transient viraemia may result from low dose vaginal inoculation (Miller *et al.*, 1994), but tissues from such animals may harbour SIV provirus despite the lack of detectable viraemia (McChesney *et al.*, 1998), and in one instance, transient viraemia following rectal inoculation resulted in subsequent virus reactivation and disease (Trivedi *et al.*, 1996).

Coreceptor usage

Like HIV, SIV requires CD4 and a coreceptor from the chemokine receptor family for infection. Both M-tropic and T-tropic strains of SIV use CCR5, although they bind to different domains of the molecule (Chen *et al.*, 1997; Edinger *et al.*, 1997a; Marcon *et al.*, 1997), while some HIV-2 strains can use CXCR4 (Hill *et al.*, 1997). Although signalling through the CCR5 receptor is not necessary for SIV infection (Edinger *et al.*, 1997a), it has been suggested that events subsequent to virus entry may be affected by coreceptor binding (Chackerian *et al.*, 1997). This is supported by the fact that the envelope glycoprotein from an M-tropic SIV strain (SIV_{smmPBj1.9}), but not from a T-tropic variant (SIV_{mac239}), was able to transduce intracellular signalling events upon binding to CCR5 (Weissman *et al.*, 1997).

Additional coreceptors also influence the tropism of SIV. For example, many SIV strains are able to replicate in the CCR5-negative CEM × 174 cell line. This is thought to be due to the fact that CEM × 174 cells express additional chemokine receptors, Bonzo (STRL33) and BOB (GPR15). Bonzo and BOB can serve as coreceptors for SIV, HIV-1 and HIV-2, and are both expressed in lymphoid tissues; BOB is also expressed in colon (Deng *et al.*, 1997). Furthermore, some SIV strains, including a neurovirulent isolate, can directly infect brain endothelial cells via a CD4-independent, CCR5-mediated mechanism (Edinger *et al.*, 1997b).

Primary, unpassaged SIV_{smm} strains are unable to use BOB as a coreceptor, indicating that some adaptation to the use of receptors other than CCR5 may occur during *in vitro* passage (Chen *et al.*, 1998). This may be analogous to the conversion of primary M-tropic HIV-1 isolates into CXCR4-dependent, T-tropic strains upon prolonged passage in human T cell lines. Overall, the molecular determinants which influence SIV usage of coreceptor molecules are less well understood than those for

HIV-1 – although they may be similar, since a single amino acid change in the V3 loop of the envelope protein of SIV_{mac239} has been shown to impair its ability to enter CEM × 174 cells, but not cells expressing CCR5 (Kirchhoff *et al.*, 1997).

Characteristics of pathogenic SIV infection

Virus dissemination and early infection

Dissemination of SIV is rapid following rectal, vaginal or intravenous exposure. The gut-associated lymphoid tissue (GALT), which includes Peyer's patches, lamina propria lymphocytes and intraepithelial lymphocytes (IELs) is the largest lymphoid 'organ' in the body and contains large numbers of highly susceptible activated CD4⁺ memory T cells, which may serve as a means for dissemination of virus to other tissues (Veazey & Lackner, 1998). Intravenous inoculation of rhesus macaques with SIV_{mac239} has been shown to lead to severe depletion of CD4⁺ T cells in the gut within 7 days of virus inoculation (Veazey *et al.*, 1998). This preceded changes in the peripheral T cell compartment, and occurred at a time when there was a much greater number of infected cells within the GALT than in the peripheral lymphoid tissues.

Rapid virus dissemination also follows mucosal virus inoculation. Infected lymphocytes and Langerhans' cells were found in the lamina propria of cervicovaginal mucosa and in T cell regions of internal iliac lymph nodes within 2 days of vaginal inoculation of rhesus macaques with the T-tropic virus strain, SIV_{mac251} (Spira *et al.*, 1996). Recent studies have sought to examine more closely the first cellular targets of SIV infection, following mucosal infection. Studies by Hirsch *et al.* (1998) revealed that the majority of virus-infected cells detected within the first few days following rectal inoculation of rhesus macaques with an M-tropic SIV variant (SIV_{smPBj}) were in fact IELs (i.e. T cells). Far fewer virus-infected macrophages were detected at this very early stage of the infection process. These results suggest that T cells but not macrophages or dendritic cells may be the most common virus targets at the early stages of infection. Even so, macrophages may still be crucial to disease pathogenesis.

Comparative studies of the early replication of wild-type M-tropic SIV (SIV_{smPBj}) and an isogenic, *vpx*-deleted virus have shown that the two viruses were readily transmitted across the rectal mucosa and were equally efficient at infecting IELs (Hirsch *et al.*, 1998). However, the *vpx*-deleted virus was unable to undergo subsequent dissemination and did not cause high level viraemia, possibly because of its inability to productively infect macrophages (Hirsch *et al.*, 1998).

Depletion of CD4⁺ T cells

Pathogenic SIV infection causes a fatal immunodeficiency syndrome characterized by progressive depletion of CD4⁺ T lymphocytes, decreased responsiveness of peripheral blood

lymphocytes to mitogen stimulation, thymic atrophy and opportunistic infections (Baskin *et al.*, 1988; Benveniste *et al.*, 1988; Letvin *et al.*, 1985). While it is clearly established that high plasma virus loads early in infection are predictive of rapidly progressive disease (Dittmer & Hunsmann, 1997; Hirsch *et al.*, 1996; Ten Haaft *et al.*, 1998; Watson *et al.*, 1997), understanding of the mechanisms for T cell depletion remains incomplete. There is good evidence that early changes in peripheral blood T cell populations are largely the result of changes in lymphocyte distribution (Schenkel *et al.*, 1999), which may occur in response to the secretion of proinflammatory cytokines by lymph node cells (Rosenberg *et al.*, 1997, 1998). Studies have also shown that there is an increase in the turnover of both CD4⁺ and CD8⁺ T cells in SIV infection (Mohri *et al.*, 1998; Rosenzweig *et al.*, 1998). However, these generalized changes do not account for the selective loss of CD4⁺ T cells, which is the hallmark of AIDS.

Activation-induced apoptosis has been evaluated as a mechanism of CD4⁺ T cell loss in AIDS. However, apoptosis occurs primarily in bystander cells, and affects both CD4⁺ and CD8⁺ T cells (Finkel *et al.*, 1995; Gummuluru *et al.*, 1996; Xu *et al.*, 1997). Apoptosis and depletion of thymic progenitors also occur early in infection of juvenile macaques, but the effect is transitory (Wykrzykowska *et al.*, 1998). Interestingly, a Nef-deleted mutant, SIV_{mac239}^{ΔNef} did not cause CD4⁺ T cell depletion in the GALT, but did cause marked activation of both CD8⁺ and CD4⁺ T cells, suggesting a critical role for Nef in T cell apoptosis (Veazey *et al.*, 1998). Consistent with this, SIV Nef has been shown to be essential for virally mediated upregulation of Fas ligand expression, and this has been proposed to represent a possible mechanism for virus immune evasion and induction of apoptosis (Hodge *et al.*, 1998; Xu *et al.*, 1997).

Since FasL expression is regulated in response to T cell activation, and is controlled in part by the transcription factor nuclear activator of T cells (NFAT), one might anticipate that the NFAT-repressing, immunosuppressive drug cyclosporin A (CsA) could provide therapeutic benefits in the context of SIV infection. Unfortunately, CsA (which has a direct antiviral effect on HIV-1 but not SIV), did not alter disease progression in SIV-infected animals, though the time of peak antigenaemia was delayed slightly (Martin *et al.*, 1997). Similarly, CsA did not protect against fatal infection of pig-tailed macaques with SIV_{smmPBj14} (our unpublished results). Thus, the relationship between virally induced immune activation, apoptosis and disease pathogenesis remains rather uncertain.

SIV-induced enteropathy

Enteropathy, characterized by malabsorption, diarrhoea and weight loss in the absence of detectable secondary pathogens, is common in SIV infection (Heise *et al.*, 1993a, b; Stone *et al.*, 1994). Proposed mechanisms include elevated production of pro-inflammatory cytokines, including IFN- γ and MIP-1 β , by activated CD8⁺ T cells in the GALT (Smit-McBride

et al., 1998), as well as the depletion of IELs, which secrete cytokines and growth factors important in maintaining the integrity of the intestinal epithelium (Mattapallil *et al.*, 1998).

SIV-induced neuropathology

SIV infection is often accompanied by encephalitis, although opportunistic infections of the brain are uncommon (Desrosiers, 1990; Murray *et al.*, 1992). Frequent neuropathological findings include perivascular infiltration of macrophages, lymphocytic meningitis and multinucleated giant cells (Desrosiers, 1990; Lackner *et al.*, 1991). However, no correlation between the severity of clinical disease manifestations, such as motor and cognitive impairments, and the degree or location of neuropathological changes has been detected (Murray *et al.*, 1992). This has made further understanding of the neuropathogenesis of SIV infection somewhat difficult. In light of this, it is not surprising to note that the mechanisms which account for neuronal injury and dysfunction in SIV-infected macaques are poorly understood, and that while a substantial body of literature exists defining potential mediators of HIV-1 neurotoxicity, relatively little work has been done in the simian model (Dewhurst *et al.*, 1996). In spite of these drawbacks, SIV has proven valuable in broadening our understanding of the interaction between primate lentiviruses and the brain.

One area of active investigation concerns the mechanism(s) of virus entry into the central nervous system. SIV is known to enter the brain within the first few weeks of virus infection, but it remains unclear how this happens. One model proposes that SIV-infected monocytes are actively recruited to the brain by upregulation of monocyto-tropic chemokines and adhesion molecules, such as VCAM-1 (Sasseville *et al.*, 1995, 1996). However, direct evidence for chemokine- or cytokine-mediated recruitment of SIV-infected cells across the blood-brain barrier has not been shown, and no correlation between the expression of VCAM-1 and brain macrophage numbers has been detected (Lane *et al.*, 1996). It is therefore possible that other mechanisms, including the direct infection of brain endothelial cells, may contribute to the entry of SIV into the brain (Edinger *et al.*, 1997*b*; Mankowski *et al.*, 1994; Strelow *et al.*, 1998).

Efforts have also been directed at identifying viral and/or host factors that affect neurovirulence. Species differences may play a role, as the severity of neurological lesions was greater in pig-tailed macaques than in rhesus macaques inoculated with the same strain of SIV (Novembre *et al.*, 1998; Zink *et al.*, 1997). Other factors which may contribute to virus neurovirulence include the virus host cell range and other virus genetic determinants. Thus, the ability to replicate in macrophages was acquired during brain passage of SIV_{mac239} (Joag *et al.*, 1995; Sharma *et al.*, 1992), but additional brain passage was required to attain neurovirulence. Evaluation of chimeric viral genomes constructed using the brain-passaged neurovirulent isolate and

the parental SIV_{mac239} clone revealed that the *env* and *nef* genes from the neurovirulent isolate were sufficient to confer neurotropism (Flaherty *et al.*, 1997; Mankowski *et al.*, 1997). The requirement for *nef* in neurovirulence may be a function of its ability to incorporate a novel unidentified Nef-associated kinase (NAK) into virus particles (Barber *et al.*, 1998; Flaherty *et al.*, 1998).

Virus and host determinants of pathogenesis

Host determinants of pathogenesis

Host determinants which influence the pathogenesis of SIV infection include genetic, immune and innate factors. For example, neonatal animals show increased susceptibility to disease induction by SIV, as compared to adult macaques (Baba *et al.*, 1995). In addition, species-specific differences in the susceptibility of macaques to disease induction by SIV_{mac/smm} have been reported, with pig-tailed macaques often showing accelerated kinetics of disease induction as compared to rhesus macaques (Hirsch *et al.*, 1995; Rosenberg *et al.*, 1991). The reasons for this remain uncertain.

Taken as a whole, the mechanism(s) which contribute to antiviral immunity, and the evaluation of novel vaccination strategies represent one of the most intensively studied areas in SIV research. The reader is referred to a number of excellent reviews on this subject, which is largely beyond scope of the present review (Almond & Heeney, 1998; Geretti *et al.*, 1998; Haigwood & Zolla-Pazner, 1998; Johnson, 1996; Lamb-Wharton *et al.*, 1997). With respect to the pathogenesis of SIV infection, and the role of host immune responses in regulating the outcome of infection, a few key points can be made. First, the temporal association between suppression of acute viraemia and emergence of detectable cellular and humoral immune responses against SIV suggests that the immune system is reasonably effective at limiting virus replication, at least in the early stages of SIV infection (Letvin *et al.*, 1994; Reimann *et al.*, 1994; Yasutomi *et al.*, 1993). Second, pre-existing immunity can dramatically alter the outcome of infection, even if sterilizing immunity is not achieved. For example, macaques treated with a variety of immunogens have been shown to be fully or partially protected from the development of immunodeficiency disease following infection with live, pathogenic SIV, and to exhibit significantly reduced steady-state levels of virus in their blood, relative to untreated controls (Desrosiers *et al.*, 1989; Gallimore *et al.*, 1995; Giavedoni *et al.*, 1993; Hirsch *et al.*, 1994, 1996; Israel *et al.*, 1994; Lohman *et al.*, 1994; Marthas *et al.*, 1990; Murphey-Corb *et al.*, 1991; Sutjipto *et al.*, 1990). Third, vaccine-induced SIV-specific CTL precursor frequency has been shown to correlate inversely with virus load after challenge (Gallimore *et al.*, 1995). This has provided support for the notion that virus-specific CTL responses are critical to the control of virus infection. Nonetheless, the requirements for protective antiviral immunity, and the relative contributions of CTL,

antibody, cytokines and chemokines to immune protection remain poorly understood.

Virus genetic variation and immune evasion

Virus genetic variation in SIV-infected macaques has been studied, in an effort to understand virus responses to host-imposed selective pressures. In contrast to HIV-1, which contains dominant linear neutralizing epitopes in the highly variable V3 loop of gp120, the V3 loop of the SIV envelope glycoprotein is well-conserved (Overbaugh *et al.*, 1991). SIV envelope gene variation occurs principally in the V1 and V4 variable domains (Almond *et al.*, 1993; Burns & Desrosiers, 1991; Overbaugh *et al.*, 1991), and conformation-sensitive antibody neutralization epitopes span both the conserved V3 region and the hypervariable V4 domain (Glamann *et al.*, 1998; Javaherian *et al.*, 1994; Kinsey *et al.*, 1996). This may allow the virus to successfully evade neutralizing antibody responses. CTL escape mutants have also been detected in SIV-infected macaques (Mortara *et al.*, 1998), as they have in HIV-1 infected humans, suggesting that multiple mechanisms may contribute to virus evasion of host immune responses, including Nef-mediated effects on antigen presentation and FasL expression (see below).

Virus genetic determinants of pathogenicity

The SIV genome, like that of other lentiviruses, contains genes that are not essential for virus replication in cultured cell lines. For SIV, these genes are: *vif*, *vpr*, *vpx* and *nef*. Vpr and Vpx are related virion-associated proteins (Yu *et al.*, 1990). Neither Vpx nor Vpr alone is essential for disease induction by the T-tropic mac239 strain of SIV (Desrosiers *et al.*, 1998; Gibbs *et al.*, 1995; Hoch *et al.*, 1995), although deletion of both *vpr* and *vpx* has been shown to result in a high degree of attenuation of SIV_{mac239} (Gibbs *et al.*, 1995). A somewhat different situation may pertain to M-tropic strains of SIV. In this case, *vpx* has been shown to be essential for efficient virus replication in macrophages (Fletcher *et al.*, 1996; Yu *et al.*, 1991), possibly because of its role in the nuclear import of viral pre-integration complexes in non-dividing cells (Fletcher *et al.*, 1996; Park & Sodroski, 1995). Furthermore, deletion of *vpx* resulted in a dramatic attenuation of the pathogenicity of the M-tropic PBJ14 strain of SIV_{smm} (Hirsch *et al.*, 1998).

Evaluation of the pathogenicity of various deletion mutants of SIV_{mac239} and other virus strains, supports the following relative ranking of viral accessory genes in terms of their contribution to virus pathogenicity (from highest to lowest): *nef* > *vpx* > *vpr*; evaluation of the role of *vif* in virus pathogenicity is complicated by the fact that *vif*-deleted viruses grow very poorly *in vitro* and are only weakly infectious for macaques (Desrosiers *et al.*, 1998). One reason for the rather modest contribution of SIV Vpr to pathogenesis may be the fact that this protein carries out only one of the two

major functional activities of its HIV-1 counterpart – namely, cell cycle arrest (Fletcher *et al.*, 1996; Park & Sodroski, 1995). Nonetheless, there is still strong selective pressure to maintain an intact *vpr* gene in SIV-infected macaques, since SIV mutants lacking a start codon for *vpr* underwent genetic reversion within 4–8 weeks of inoculation into rhesus macaques (Lang *et al.*, 1993).

Determinants of virus pathogenicity may reside not only in the protein-encoding regions of the viral genome, but also in the regulatory elements of the virus. The SIV_{mac239} long terminal repeat (LTR) contains transcriptional regulatory elements which include a TATA box, four Sp1-binding sites and one NF- κ B-binding site, as well as additional upstream elements not found in HIV-1. All of these elements were found to be dispensable for virus replication in PBMCs (Ilyinskii & Desrosiers, 1996), and SIV_{mac239} mutants lacking the Sp1 and NF- κ B sites were able to cause immunodeficiency disease in rhesus macaques. However, none of these infected animals developed granulomatous pneumonia or encephalitis, possibly because of impaired virus replication in macrophages (Ilyinskii *et al.*, 1997). This would be consistent with *in vitro* findings, showing that the Sp1 or NF- κ B sites within the LTR are required for replication of macrophage-competent derivatives of SIV_{mac239} in macrophages (Bellas *et al.*, 1993; Ilyinskii & Desrosiers, 1996).

Role of Nef in SIV pathogenesis

The paramount importance of Nef in SIV pathogenesis has been apparent since Kestler *et al.* (1991) showed that macaques infected with a *nef*-deleted SIV_{mac239} mutant remained disease-free and maintained greatly reduced virus loads. For the purposes of the present discussion, we will focus our attention on functions of Nef that may play a role in virus pathogenicity, and on areas that remain controversial; readers are referred to a recent review article for a more complete discussion on Nef (Harris, 1996).

SIV Nef is a 32–34 kDa myristoylated protein that is incorporated into virus particles (Bukovsky *et al.*, 1997; Flaherty *et al.*, 1998). SIV Nef shares structurally conserved central domains with HIV-1 Nef, and diverges at its amino- and carboxy-termini (Iafrate *et al.*, 1997). In addition, HIV-1 and SIV Nef are functionally interchangeable within the context of chimeric viruses, when assessed for their ability to enhance the infectivity and replication kinetics of such viruses in cultured macaque and human PBMCs (Sinclair *et al.*, 1997). Nonetheless, important differences in the proteins exist, as revealed by the fact that chimeric simian–human immunodeficiency viruses containing SIV *nef* were more pathogenic in macaques than those containing HIV-1 *nef* (Shibata *et al.*, 1997).

Conserved functions of SIV and HIV-1 Nef include the ability to enhance the infectivity of virus particles, the alteration of lymphocyte activation events (Alexander *et al.*, 1997; Iafrate *et al.*, 1997) and the downmodulation of CD4

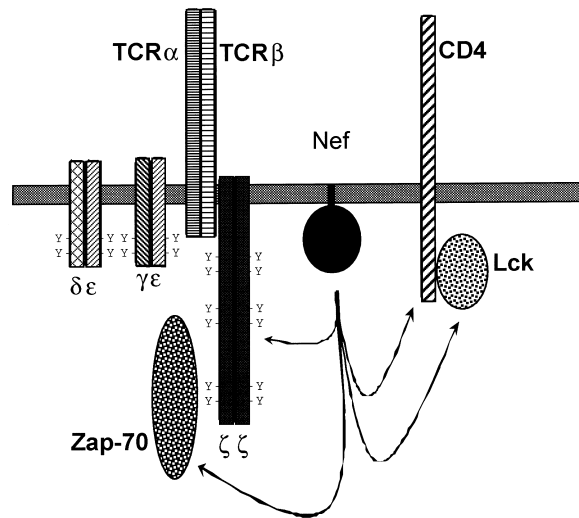


Fig. 2. Schematic diagram of the interplay between SIV Nef and components of the T cell signalling machinery. The TCR is shown, with the various subunits denoted; SIV Nef is known to interact directly with the zeta chain (ζ) (Bell *et al.*, 1998; Howe *et al.*, 1998). TCR-associated tyrosine residues which comprise ITAM motifs that are involved in TCR-mediated signalling events and the recruitment of the TCR-associated, T cell PTK Zap-70 are also shown (Y). As indicated by the arrow, a unique, ITAM-containing SIV *nef* allele (from SIV_{smmPBj14}) has been shown to interact directly with Zap-70 (Luo & Peterlin, 1997). In addition, Nef is known to interact with CD4, leading to the downregulation of cell surface expression of this molecule, with displacement of the CD4-associated tyrosine kinase, Lck (Foster *et al.*, 1994; Garcia & Miller, 1991; Hua & Cullen, 1997; Iafrate *et al.*, 1997). Finally, SIV and HIV-1 Nef have also been shown to interact directly with Lck (Baur *et al.*, 1997; Collette *et al.*, 1996).

from the surface of infected T cells (Foster *et al.*, 1994; Garcia & Miller, 1991; Hua & Cullen, 1997; Iafrate *et al.*, 1997). While the contribution of these various functions to virus pathogenesis remains uncertain, considerable progress has been made in defining the biochemical pathways which may contribute to the various effects of Nef.

Recent results suggest that HIV-1 and SIV Nef may promote internalization of CD4 at least in part through a specific interaction with adaptor protein (AP) complexes involved in the recognition of protein sorting signals by the clathrin-dependent machinery (Greenberg *et al.*, 1997; Le Gall *et al.*, 1998; Piguet *et al.*, 1998). It is of interest to note that the ability of SIV Nef to bind to AP proteins is dependent upon a YxxL motif, which resembles tyrosine-based endocytotic sorting signals (Piguet *et al.*, 1998). This motif is absent in HIV-1 Nef, indicating that HIV-1 Nef must interact with the AP complex via a different, but unknown, mechanism.

SIV and HIV-1 Nef also interact specifically with a protein that appears to represent the catalytic subunit of the human vacuolar ATPase (Lu *et al.*, 1998). This may provide an alternative mechanism whereby Nef can target proteins to the endosomal pathway, and it suggests that Nef may alter cell surface protein expression in several ways. Indeed, Nef downmodulates the cell surface expression of MHC class I

molecules, in a manner that may not depend on its interaction with AP complexes (Greenberg *et al.*, 1998; Le Gall *et al.*, 1998; Schwartz *et al.*, 1996). However, the *in vivo* relevance of this finding to virus pathogenesis is unclear—even though HIV-1 Nef-mediated downregulation of MHC has been shown to protect cells against CTL-mediated lysis *in vitro* (Collins *et al.*, 1998).

Additional properties associated with SIV Nef include an essential role in virally mediated induction of Fas ligand on T cells (Hodge *et al.*, 1998; Xu *et al.*, 1997), as well as an association with a variety of cellular kinases (Harris, 1996), including the CD4-associated protein tyrosine kinase (PTK) Lck (Baur *et al.*, 1997) and, in the case of the SIV_{smmPBj14} Nef protein, the T cell receptor (TCR)-associated PTK, Zap-70 (Luo & Peterlin, 1997) (Fig. 2). In many cases, the binding sites for these kinases have been mapped to short regions of the Nef protein. For example, the region of SIV_{smmPBj14} Nef which binds to Zap-70 is a tyrosine-based ITAM motif, which is essential for the acutely pathogenic and lymphoproliferation-inducing phenotype of this virus (Du *et al.*, 1995, 1996; Luo & Peterlin, 1997; Saucier *et al.*, 1998).

A highly conserved SH3-binding domain (PxxP) that is found within both SIV and HIV-1 Nef has been shown to interact with cellular kinases, including the Src family of protein kinases and a cellular threonine/serine kinase, designated NAK (Collette *et al.*, 1996; Manninen *et al.*, 1998; Saksela *et al.*, 1995). NAK may represent a member of the p21-activated kinase (PAK) family of kinases, and its importance in virus pathogenesis has been examined through the use of mutated SIV genomes lacking the PxxP motif. Studies conducted by two different groups using identical SIV_{mac239} mutants (PxxP → AxxA) resulted in very divergent conclusions, but the data are in many respects similar. Lang *et al.* (1993) found that the AxxA mutant virus caused disease and death in 2/2 inoculated animals. They also found evidence of reversion of the AxxA mutation in 5–10% of viral genomes present in tissues collected from these animals. Khan *et al.* (1998) also saw disease induction and death in macaques that they inoculated with their AxxA mutant, but they reported a much higher level of genetic reversion of the AxxA mutation (up to 70%, in 5/6 chronically infected animals), and a functional restoration of NAK-binding activity. Thus, it would appear that there is strong selection for the PxxP motif *in vivo*. In addition, it is likely that this motif may be required for the induction of immunodeficiency disease, since the virus loads reported by Lang *et al.* (1993) were very high, meaning that even a low proportion of revertant virus (5–10%) might have been sufficient to cause disease.

The effects of Nef on T cell signalling are, if anything, even more disputed and uncertain. A fairly substantial body of literature exists demonstrating the inhibition of T cell activation pathways by HIV-1 and SIV Nef (Baur *et al.*, 1994; Collette *et al.*, 1996; De & Marsh, 1994; Graziani *et al.*, 1996; Greenway *et al.*, 1995; Iafrate *et al.*, 1997; Niederman *et al.*,

1992). On the other hand, some experiments suggest that Nef actually activates T cells (Alexander *et al.*, 1997; Du *et al.*, 1995; Hanna *et al.*, 1998; Lindemann *et al.*, 1994; Luo & Peterlin, 1997; Skowronski *et al.*, 1993). The question of the effects of Nef on T cells is further complicated by recent findings that SIV and HIV-2, but not HIV-1, Nef can interact with the zeta chain of the T cell receptor (TCR ζ) (Bell *et al.*, 1998; Howe *et al.*, 1998) (Fig. 2).

Conclusions and future directions

SIV-macaque models have proved to be highly useful in probing the pathogenic mechanisms that underlie the progression of acquired immunodeficiency disease. While considerable progress has been made, many important questions remain unresolved. These include the following: (1) why do viruses that are apathogenic in their natural hosts become virulent in new hosts (such as humans)? (2) What factors influence the level of virus replication and the 'set point' of viraemia? (3) Why does the immune system ultimately fail in its efforts to contain virus replication, and can therapies designed to boost antiviral responses provide therapeutic benefit? (4) Can we more accurately map the virus determinants for pathogenesis, and can this information be used to develop new antiviral drugs (for example, drugs that might target viral accessory genes or drugs that specifically interfere with the ability of the virus to cause neurologic disease)? (5) Finally, can we use the SIV-macaque model to develop a safe and effective vaccine for AIDS?

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