Abstract
The development of anti-virals has blunted the AIDS epidemic in the Western world but globally the epidemic has not been curtailed. Standard vaccines have not worked, and attenuated vaccines are not being developed because of safety concerns. Interest in attenuated vaccines has centered on isolated cases of patients infected with HIV-1 containing a deleted \textit{nef} gene. Nef is a multifunctional accessory protein that is necessary for full HIV-1 virulence. Unfortunately, some patients infected with the \textit{nef}-deleted virus eventually lose their CD4$^+$ T cells to levels indicating progression to AIDS.

This renders the possibility of an attenuated HIV-1 based solely on a deleted \textit{nef} remote. In this review we discuss the knowledge gained both from the study of these patients and from in vitro investigations of Nef function to assess the possibility of developing new anti-HIV-1 drugs based on Nef. Specifically, we consider CD4 downregulation, major histocompatibility complex I downregulation, Pak2 activation, and enhancement of virion infectivity. We also consider the recent proposal that simian immunodeficiency viruses are non-pathogenic in their hosts because they have Nefs that downregulate CD3, but HIV-1 is pathogenic because its Nef fails to downregulate CD3. The possibility of incorporating the CD3 downregulation function into HIV-1 Nef as a therapeutic option is also considered. Finally, we conclude that inhibiting the CD4 downregulation function is the most promising Nef-targeted approach for developing a new anti-viral as a contribution to combating AIDS.

Introduction
The brutal attack on humanity by HIV-1 has proven to be distressingly difficult to counter. The best results at blunting the epidemic have been the development of anti-retrovirals (ARVs) that inhibit crucial HIV-1 functions. Unfortunately, the unique ability of HIV-1 to mutate and adapt [1,2] requires multiple drug treatments that are limited in their application by their side effects and their expense. Topically applied microbicides offer the possibility of prevention, but similar problems of toxicity, expense, and effective application apply here as well as with ARVs [3,4]. Vaccines have been a total failure and future prospects are dim [5-8].

Well into the third decade of HIV-1 research the likelihood of finding an Achilles' heel for HIV-1 is remote. The virus is too highly adapted from its successful 70 year contest with the human immune system [9,10]. Accumulating small victories are the probable long term course for significantly curtailing the epidemic. Effective microbicides are desperately needed for vaginal pre-exposure prophylaxis and post-exposure prophylaxis. New ARVs
that inhibit an increasing number of viral processes are critical for treating already infected individuals. ARVs are potentially useful in prophylaxis as well. In this case topically applied drugs would ideally be different from drugs used for treating HIV-1 since topical application could lead to resistant strains of HIV-1 [3,4]. Therefore, all possible targets for countering HIV-1 need to be considered. Given its central role in HIV pathogenesis, in this article we consider Nef as a potential anti-viral target for preventing or at least delaying pathogenesis.

Ironically, the overwhelming focus for a Nef-based therapeutic intervention has been the investigation of a nef-deleted attenuated virus vaccine. This interest resulted from a small number of cases of long term non-progressors (LTNP) whose viruses have irretrievable deletions in the nef gene [11-14]. Unfortunately, some individuals infected with the nef-deleted virus are slow progressors (SP) rendering a nef-deleted attenuated vaccine too dangerous. We will not review this aspect of the Nef field in detail since an excellent review has been recently published on the most important of these cases - the Sydney Blood Bank Cohort (SBBC) [15]. We will discuss several aspects of SBBC and other cases that shed light on the role of Nef in the development of HIV-1 disease.

The lack of disease progression in patients whose HIV-1s are nef-deleted, defines Nef as a pathogenic factor. Whether Nef acts as a generalized enabler of high levels of replication or is directly pathogenic remains unresolved. In either case it would seem logical to investigate blocking Nef function in order to lessen the severity of HIV-1 disease. Though the idea of Nef as a target for drug intervention in HIV-1 disease has rarely been considered [16,17], Betzi et al. have recently identified the first compounds that target Nef [18]. The major problem is the daunting complexity of Nef’s multiple functions. Accordingly, we will discuss four intensely studied Nef activities and assess possible roles for each function in pathogenesis. These are CD4 downregulation, major histocompatibility complex I downregulation, activation of p21-activated protein kinase (Pak2), and enhancement of virion infectivity [19]. Each function is genetically separable from the others and therefore represents a distinct target for inhibiting Nef [20,21]. That each of these four functions is mechanistically distinct implies that an anti-Nef drug will not be able to debilitate Nef in general, but probably block only one or two. This makes it imperative to determine the Nef function most relevant to pathogenesis. In addition, we will discuss the possibility of a radical new approach to viral pathogenesis based on the recent model of simian and human lentivirus pathogenesis being controlled by the downregulation of CD3 by Nef [22]. Finally, we will conclude that an attenuated virus vaccine based solely on a Nef deletion is still remote, and that CD4 downregulation is the most promising target for attacking HIV-1 through Nef.

**Nef and disease progression**

Nef was first shown to be a major determinant of primate lentivirus pathogenicity when it was demonstrated that a large deletion in the nef gene greatly reduces the severity of simian immunodeficiency virus (SIV) induced disease in rhesus macaques. Furthermore, following intravenous injection of macaques with an SIV encoding a nef gene with a premature stop codon, the nef open reading frame (ORF) was rapidly restored. This demonstrated that there was significant selective pressure to express the SIV Nef protein [23]. HIV-1 Nef also has a key role in pathogenesis. There are four separate examples of LTNP’s infected with nef-deleted HIV-1. As indicated above, the best studied is the Sydney Blood Bank Cohort [15]. Infection occurred in the short time frame between the appearance of HIV-1 in Australia and the institution of HIV-1 blood testing. A single donor contributed multiple units of contaminated blood. Red cells or platelets from that blood were given to ten patients with eight of these recipients becoming infected [24]. The high rate of infection is comparable to the rate of transfusion-associated HIV-1 infection in general which is approximately 60% [25]. This is a striking result since the blood contributed by the donor in the SBBC carried low levels of nef-defective virus. Clearly, Nef is not required for transmission by blood, but is a crucial factor for disease development. It is important to note the rarity of blood transfusion related infections by nef-deleted HIV-1s. Only one other case has been reported - a hemophiliac infected by a Factor VIII preparation contaminated with HIV-1 [12] compared to the 12,000 transmissions through the blood supply in the United States alone [25].

Though Nef is not required for blood to blood transmission it appears to be an important factor for sexual transmission. This is shown by the fact that there are only four reported cases of non-transfusion related infections by Nef-defective virus. These are the donor in the SBBC cohort who was a sexually active homosexual male [11], a homosexual male from Italy [14], and a male who contracted a nef defective virus heterosexually in Thailand and then transmitted the virus to his wife [13]. The virus from all SBBC recipients and three of the four just mentioned sexual transmissions exhibit a surprising convergence. They all have two similar defects in the nef gene. First, the coding region of Nef from near the initiation codon to near the 5’ end of the polypurine tract (ppt) is deleted. Second, there is a large deletion from just downstream of the ppt to the end of Nef but not into the major promoter elements of U3. The simple explanation for these genetic convergences is that the two described regions have no major functions other than to code for Nef, and in the
absence of Nef function a slight advantage is accrued to replication by completely deleting them. The one exception was the male who contracted AIDS in Thailand. This subtype E virus exhibited a wide range of Nef sequences from intact to large deletions including the ppt. Blood samples from the time of HIV-1 transmission to his wife are not available [13] to explain how she came to be infected with the double deleted Nef just described.

Death as a result of AIDS has not been observed in any of the people infected by Nef-deleted virus, but in some cases it was apparent that disease was advancing. There are 6 SBBC patients (C49, C64, C135, C54, C98, D36) whose HIV-1 infections have been extensively documented. Three recipients- C49, C64, and C135- lived over 20 years without any sign of disease. Virus was not detectable in blood from these patients, and they exhibited minimal antibody responses [26]. Therefore, these patients are "elite" long term non-progressors. Three additional patients had detectable viral loads and an eventual decline in CD4+ T cells in blood after 17 or more years of being infected. C54 died of non-AIDS causes before the decline in CD4+ T cells necessitated anti-retroviral drugs. C98's CD4+ T cells declined to nearly 200 cells/ml, and received anti-viral therapy for 16 months before dying of non-AIDS causes. The blood donor of the cohort, D36, declined after 18 years to 160 CD4+ T cells/ml and developed HIV-associated dementia [27]. At the point of commencing therapy his plasma HIV-1 RNA was 9900 copies/ml and there were over 750,000 copies/ml in cerebrospinal fluid. One month after receiving therapy plasma viral load was undetectable and CD4+ T cell levels increased [28].

These last three patients are best described as slow progressors (SP). Another SP was the above mentioned hemophiliac infected through a contaminated Factor VIII preparation [12]. This individual was one of 7 LTNP's out of a study group of 128 infected hemophiliacs [29]. PCR screens for full length Nef genes yielded only this patient as having a doubly truncated Nef. For about 10 years post-infection his CD4+ T cell counts were stable, but after another 3 years his CD4+ T cell count fell to 261 and HAART was initiated [30]. An additional case of a LTNP is an Italian homosexual whose CD4+ T cell levels have not altered in 20 years of infection and whose viral loads have been steady at the extremely low value of about 200 copies/ml. As previously mentioned Nef sequences derived from this person's virus contained two deletions in Nef upstream and downstream of the ppt [14]. Nine years later the entire HIV-1 genome from this individual was sequenced. Surprisingly, sequence of the env gene, but not gag, pol, vif, vpr, tat or rev, also showed large deletions [14,31]. Large deletions in genes other than nef have not been seen in the SBBC [32]. Finally, the husband and wife that are infected with a nef-deleted subtype E virus also appear to be LTNP's. They have not shown any signs of disease progression but they may not have been infected longer than 10 years [13].

Summarizing these studies it is evident that in vivo Nef is a critical factor in HIV-1 replication, but it is not absolutely necessary. Despite patients infected with nef-defective HIV-1 having little or no virus in their blood some did progress towards HIV-1 disease. What percentage of HIV-1 infected individuals have nef-deleted virus is difficult to estimate since without disease progression many cases could go undetected. If the percentage were anything other than extremely low, one would certainly expect many more cases to have been uncovered. The same argument applies to the transmission of nef-defective HIV-1 sexually. For example, the HIV-1 positive status of the husband and wife pair was revealed as a result of testing during pregnancy [13]. Therefore, it would seem that Nef is not only a pathogenic factor but also a sexual transmission factor.

Which Nef functions are required for pathogenesis?
Nef is a small protein devoid of enzymatic activity. It is polymorphic in length (200–215 amino acids) with the most common length being 206 [33]. It is myristoylated and mainly localized in the paranuclear region with reduced expression at the plasma membrane. It serves as an adaptor protein to divert host cell proteins to aberrant functions that amplify viral replication [34,35]. Four in vitro activities of HIV-1 Nef have been extensively documented. They are: 1) Nef downregulates cell surface levels of CD4 [36-40]; 2) Nef downregulates cell surface levels of major histocompatibility class I (MHCI) molecules [41-45]; 3) Nef mediates cellular signaling and activation [46-49]; and 4) Nef enhances viral particle infectivity by CD4 independent mechanisms [50-55].

Each of these four Nef functions could serve as contributors to Nef's elusive role in replication and pathogenesis. Several reports have suggested the importance of removing CD4 from the surface of infected cells for the production of infectious HIV-1 particles [39,56]. Without this Nef function host cell CD4 can bind to Env during virion budding and interfere with the production of fully infectious particles. Also, Nef's ability to down-modulate MHCI molecules could facilitate HIV-1 immune evasion and thus enhance virus replication [57,58]. A third possible Nef-mediated enhancement of pathogenesis is cellular activation of cell signaling pathways that could enhance replication in partially stimulated T cells. For example, if Nef functions in vivo to elevate the activation level of certain partially activated T cell populations then viral production in those cells would be increased [59,60]. Of particular interest in this regard are the memory T cells in
the gut that are early targets of HIV-1 and SIV infection, even though they lack expression of classic T cell activation markers [61-63]. Finally, the well documented Nef-dependent enhancement of the infectivity of viral particles would be expected to accelerate the spread of virus in vivo. This function of Nef is distinct from the role that CD4 downregulation can play in the production of competent HIV-1 virions. These four Nef functions will now be discussed in greater detail.

A. CD4 down-modulation by Nef

The first and most extensively characterized function of Nef is its ability to dramatically reduce the steady state levels of CD4 on the cell surface [38,64]). Human CD4 is downmodulated by Nefs from HIV-1 groups M, N, and O, and simian immunodeficiency virus from chimpanzees (SIVcpz) [65], in multiple mammalian cell types [37,66], and even in Drosophila S2 cells [67]. As mentioned above the major role for this Nef activity may be in overcoming the detrimental effects of high cellular CD4 expression in the producer cell [39,68,69].

Nef-induced CD4 down-modulation involves the internalization of surface CD4 followed by degradation via the endosomal/lysosomal pathway (Figure 1, Red). Consistent with this mechanism Nef localizes to clathrin-coated pits [70] and increases the number of CD4 containing clathrin coated pits [71]. Inhibition of lysosomal acidification blocks Nef induced CD4 degradation, without restoring CD4 surface expression [72-74]. Moreover, Nef induced CD4 downmodulation is blocked by transdominant-negative dynamin-1 co-expression [75], as well as, pharmacological inhibitors of clathrin coated pit mediated endocytosis [74].

The heterotetrameric clathrin-associated adaptor protein 2 (AP-2) is a key molecular mediator of Nef induced CD4 downmodulation [76], but other aspects of CD4 downregulation remain unclear. Unlike CD4 downmodulation by phorbol esters, Nef-induced downmodulation is independent of the phosphorylation of serine residues in the CD4 cytoplasmic tail [38]. Data suggest that Nef may act as a connector between CD4 and the cell’s endocytic machinery [40], by binding the membrane proximal segment of the cytoplasmic domain of CD4 [37,38,77]. Furthermore, NMR analysis confirms that the membrane proximal segment of CD4 is necessary for a direct interaction with Nef [78]. Nef residues W57 and L58 are predicted by NMR to be critical in this interaction and have also been functionally demonstrated to be important for CD4 downmodulation [79]. The possible significance of this proposed interaction between Nef and the cytoplasmic tail of CD4 is obscured by the fact that it is weak, but the interaction of p56Lck and CD4 is strong and the p56Lck-CD4 complex is not subject to rapid endocytosis [80,81]. Further, it is unlikely that Nef binds directly with p56Lck intracellularly [82], even though Nef has been shown to induce endosomal accumulation of Lck [83]. This latter effect of Nef on Lck does not appear to be related to CD4 downregulation since the L164A/L165A mutant of Nef alters the intracellular distribution of Lck but fails to downregulate CD4 [83,84]. An alternate model to the direct binding of Nef to the cytoplasmic tail of CD4 has been proposed by Coleman et al. in which Nef disregulates endosomal trafficking [85].

In contrast to the poorly defined direct interaction of Nef with the cytoplasmic tail of CD4 the direct interaction of Nef with AP-2 has been described in detail [76]. AP-2 binds to the just mentioned dileucine motif in Nef which is found in a structurally flexible loop that extends from amino acids 148 to 180 [86]. The dileucine motif in Nef exhibits a canonical 160EXXXLL165 sequence but it is not sufficient to account for the binding of Nef to AP-2. Also required are two acidic residues within the loop, 174(E/D)175. Mutation of either the dileucines or the diacidic residues to alanines disables Nef binding to AP-2 in yeast three hybrid assays (Nef/AP-2-α/EPOR) and the CD4 downregulation function. In the absence of the diacidic residues there is weak binding by the dileucine motif because of a suboptimal sequence for the XXX residues (i.e. N, T and S). Replacing 161NTS163 within the Nef dileucine motif with residues from the AP-2 interacting protein, tyrosinase, gives 160FRPL164 which even in combination with the 174AA175 mutation binds strongly to AP-2. The arrangement of a weak dileucine motif which is apparently stabilized by nearby acidic residues may be peculiar to Nef. This led Lindwasser, et al. to suggest the Nef/AP-2 interaction as a possible target for anti-virals to counter the pathological effects of HIV-1 [76]. The possibility that blocking CD4 downregulation could have a positive impact on HIV-1 pathogenesis is supported by the example of an LTNP infected by a HIV-1 with a uniquely defective Nef. Carl et al. reported a non-progres- sor (12 years without a decline in CD4+ T cells, but relatively high viral loads of 15,000 to 55,000 copies/ml) with a small deletion in Nef and a compensating duplication [87]. The virus in this patient had a deletion of 36 base pairs (amino acids 26–37) and a 33 base pair duplication (amino acids 43–53). In vitro studies demonstrated that the deletion by itself inactivates CD4 downregulation, enhancement of infectivity, MHCI downregulation, and partially destabilizes the protein. Incorporating the duplication into the deletion bearing Nef gave a partially functional protein that had restored enhancement of infectivity, MHCI downregulation, and protein expression but remained defective for CD4 downregulation. The suggestion from this one patient is that an HIV-1 lacking a Nef functional for CD4 downregulation is greatly reduced in its pathogenic potential. Therefore, Nef-mediated CD4
downregulation appears to be a potential target for anti-viral intervention, except that the flexible structure of the loop containing $^{160}$EXXXL$^{165}$ and $^{174}$E(D)$^{175}$ may not allow for the modeling of small molecules with high affinity and specificity [18]. Therefore, the potentially unique Nef-binding surface of AP-2 may be a better target.

B. MHC class I down-modulation by Nef

Another well conserved property of Nef is its ability to downmodulate MHC class I molecules [44]. As Nef is expressed early after infection, Nef induced downmodulation of MHC class I molecules could enable the infected cell to evade destruction by the immune system during active viral replication. In support, it has been demonstrated that Nef expression reduces the susceptibility of HIV infected cells to cytotoxic T lymphocyte (CTL) mediated lysis in vitro [57,58]. Therefore, determining the mechanism by which Nef downregulates MHC-I has received a high priority. Early aspects of this field have been reviewed [88].
A long standing model proposed by Thomas and co-workers [89,90] has been recently revised [41]. In this model Nef initiates MHCI downregulation by interacting with PACS-2. The Nef/PACS-2 complex localizes to the trans-Golgi network (TGN) where PACS-2 is displaced and Nef binds to a src family kinase (SFK). The SFK would then bind and phosphorylate ZAP70/Syk on tyrosine enabling ZAP70/Syk to bind the SH2 domain of phosphatidylinositol 3-kinase (PI3K). The resulting activation of PI3K would lead to elevated PIP₃, stimulation of the guanine nucleotide exchange factor ARNO, and GTP loading of ARF6. At this point the rate of MHCI endocytosis is accelerated. For the increased rate of MHCI internalization to be effective in reducing MHCI cell surface levels Nef must also block the recycling of MHCI back to the plasma membrane. The revised model does not define an interaction between MHCI and Nef, but the suggestion was made that Nef induces PACS-1 to interact with the cytoplasmic tail of MHCI [41]. However, a ternary complex between Nef, the cytoplasmic tail of MHCI and AP1 has been recently demonstrated separately by the Collins and the Guatelli laboratories [43,45]. This complex appears to activate a cryptic tyrosine sorting signal in the cytoplasmic tail of MHCI and diverts newly synthesized MHCI molecules from their transit to the plasma membrane to an internal compartment in the paranuclear region [43,45,91]. Nef appears to be acting as a facilitator since the cytoplasmic tail of MHCI does not bind to AP-1 [43,45]. How the model of Thomas and co-workers can be adapted to include this complex is as yet unresolved (Figure 1, Yellow). It is interesting to note that this ternary complex engages Nef in a novel interaction with MHCI cytoplasmic tail and AP-1 which makes it potentially appealing for targeting by an anti-viral.

However, it should be noted that Nef does not render infected cells completely protected from immune surveillance as there is a strong CTL response to HIV antigens [92]. Therefore, it appears that the downregulation of MHCI by Nef fails to block the cytotoxic T cell response to the virus, but the immune response is either misdirected or HIV-1 is able to escape by mutation or both [93]. At this point the concept of viral evolution and its relationship to viral pathogenesis should be considered. That there are constraints placed on the virus by the cytotoxic T cell response is clear if the virus mutates to avoid the response [94,95]. This does not necessarily imply that the targeted HIV-1 or the HIV-1 bearing escape mutation(s) are different in pathogenic potential. In fact, Brumme et al. [94] found an inverse relationship between the number of apparent escape mutations in Nef and the level of CD4+ T cells in the blood. In other words, virus with a Nef containing 11 or more escape mutations was more pathogenic than virus with a Nef containing 0–2 mutations. This distressing finding likely reflects Nef having success-fully evolved to readily side-step the vast majority of CTL responses. Nef has 63 very highly conserved residues out of 206 (99% identity), but they are scattered throughout the protein, so that no more than five are in a row [33]. As a result, susceptible epitopes in Nef mutate at variable residues to effectively escape CTLs, but Nef function is not affected.

A relatively small number of HLA epitopes in HIV-1 genes other than Nef have been reported that do involve escape mutations that reduce virulence. These epitopes have been suggested as the basis for a therapeutic vaccine [95]. Although Nef amino acid sequences are doubtful contributors to the proposed vaccine an inhibitor of Nef’s ability to downregulate MHCI could enhance the effectiveness of such a vaccine. In this regard the Italian male infected with a virus lacking nef subsequently evolved a virus with both a deleted nef and env. Calugi et al [31] interpreted this finding as an inability of the Nef deleted virus to protect itself from CTL attack. Unfortunately, this patient appears to be unique as the SBBC researchers did not find evidence of the development of deletions in other genes [32].

C. Cellular activation and signaling by Nef

Disease progression may be directly associated with T cell activation [96,97]. It is also possible that Nef may regulate cellular activation through several kinases including Pak2 [48,49], and Hck [82,98]. Pak2 is the well characterized Nef-activated kinase. It has been demonstrated that Nef activation of Pak2 leads to merlin phosphorylation at serine 518 though it has yet to be demonstrated that HIV-1 infection is in anyway dependent on merlin phosphorylation [46]. The obvious suggestion of this result that Nef regulates the actin cytoskeleton function is appealing, but the mechanism is controversial [99-102].

Substantial agreement exists that Nef forms a complex with Pak2 (Figure 1, Orange) [65,100,103,104]. Nef not only complexes with Pak2 but also induces Pak2 activation [49,105]. The ability of Nef to activate Pak2 in multiple HIV-1 subtypes suggests a key role for this Nef function [33,106]. An interaction domain that is responsible for Pak2 activation has been observed to include residues 89 and 191 [33]. Lesser contributions are made by residues 85 and 188 [103,106,107]. Remarkably, H89 and F191 are highly conserved in subtype B Nefs, but in subtype E Nefs F89 and R191 are highly conserved instead [33]. Since subtype E Nefs are active for Pak2 activation it appears that at least two different interaction domains are functional in HIV-1 Nefs. By substituting all four just mentioned residues in a subtype B Nef with residues that predominant in subtype E Nefs (L85F, H89F, R188A, and F191R) the subtype E Pak2 interaction surface can be created in a subtype B background. The quadruple mutant is fully functional though intermediate forms are generally
defective [33]. The significance of these alternate Pak2 activation domains remains to be determined. However, the presence of different structures to achieve the same function strongly suggests that maintaining the ability to activate Pak2 enhances viral fitness. The importance of Pak2 activation has been questioned on the basis of ex vivo experiments [108]. One possibility is that Pak2 activation may be important for transmission or early in infection. However, only limited evidence currently exists to support this hypothesis [21]. Regardless, the structural fluidity of Nef’s Pak2 interaction surface could make this Nef interaction difficult to target with anti-virals. Investigations to uncover the common features of the alternative activation domains may clarify these issues.

Nef also activates the myeloid lineage specific tyrosine kinase, Hck (Figure 1, Pink). Co-expression of Nef and Hck in Rat-2 fibroblasts leads to cellular transformation [109]. Moreover, Nef tightly binds to the Hck SH3 domain in vitro and activates its kinase activity [110]. In Rat-2 cells enforced dimerization of Nef enhances Hck activation [98]. Nef has also been shown to modestly activate endogenous Hck and, in turn, the Stat3 transcription factor in myeloid cells [111]. Interestingly, Hck is the only cellular activity of Nef known to not require Nef myristoylation [111]. The Hck/Nef interaction is mediated by an SH3 binding domain in Nef 72-PQVPLR77 which may be too similar to cellular SH3 interactions to be readily targeted by an anti-viral. On the other hand, one distinctly virus-specific interaction would be Nef dimerization which may be required for Hck activation intracellularly. In fact, Nef spontaneously forms dimers and trimers [112]. This may occur when the myristate moiety is buried in a cellular membrane. Disengagement from membrane appears to result in the myristoylated N-terminus of Nef self-associating with a hydrophobic patch on the surface of the structured core of Nef. In this latter conformation Nef is a monomer. Therefore, Nef lacking its N-terminal myristate may dimerize and activate Hck. The relevance of Hck activation for pathogenesis is unknown, but if Nef dimerization and trimerization are of pathological significance it could be a novel target for anti-virals.

D. Enhancement of HIV-1 infectivity

Early work on this topic has been previously reviewed [88]. Currently, there are three models of how Nef enhances the infectivity of HIV-1 as measured in single infection assays [50,54,113,114]. In these assays virus is produced in cells that do not express CD4 and the normalized infectivity is determined on indicator cell lines. Therefore, this effect represents increased infection efficiency for HIV-1 virions, and is distinct from Nef-dependent enhanced particle egress from infected macrophages [115]. Campbell et al. noted that the disruption of the actin cytoskeleton in the target cell complemented the defect in infectivity in Nef minus virions (Figure 1, Blue). These authors concluded that cortical actin represents a barrier to infection and that the expression of Nef in the producer cell is able to overcome this barrier [54]. In an alternate model Pizzato et al. suggest that there is an unknown cellular protein other than CD4 that blocks the function of Env in the virion. Nef enhances infectivity by downregulating this unknown protein in the producer cell and blocking its incorporation into the virion [50]. It is interesting to note that the Nef dileucine motif (164LL165) that is required for CD4 downregulation is also required for enhancement of infectivity [116]. However, the diacidic motif (174DD175) that is necessary for Nef to bind AP-2 is not. This genetically distinguishes enhancement of infectivity from CD4 downregulation. In this case it appears that Nef’s canonical dileucine binding motif involving E160 (160EXXXLL165) associates with AP-1 and AP-3 [85,116,117]. It is not yet known if these results are related to the above mentioned results of Pizzato [50]. Finally, Nef may protect the viral core from post-fusion degradation to allow reverse transcription to proceed [113,114]. This mechanism is consistent with the active degradation of HIV-1 virions that occurs upon entry [118]. Despite the attractiveness of a drug that reduces the inherent infectivity of HIV-1 virions the prospects for inhibiting Nef-mediated enhancement of infectivity are presently remote.

E. Downregulation of CD3 as a mechanism of attenuating viral pathogenesis

Schindler et al. have proposed a surprising explanation for the lack of pathologic effects of most primate lentiviruses in their hosts in contrast to the virulence of HIV-1. Specifically, it was proposed that most simian viruses self-limit their inherent pathogenicity [22]. For example, sooty mangabeys do not develop AIDS from their own SIV [119,120]. Schindler et al suggest this is the result of the SIV from sooty mangabeys (SIVSM) having a Nef that downregulates CD3 which prevents activation of the infected T cell and subsequent activation induced cell death. The authors further propose that the downregulation of CD3 evolved as a mechanism to maintain virus persistence in the presence of an intact host immune system. The CD3 downregulation function was lost in chimpanzee immunodeficiency virus (SIVCbx) prior to the infection of humans. Since HIV-1 Nef does not downregulate CD3 humans progress to AIDS as hyperactivation slowly destroys the immune system. The therapeutic implications of this concept are staggering since one must assume that during the course of natural SIVSM infection there are mutations in nef that abrogate CD3 downregulation. The unanswered question is how these potentially pathogenic SIVSM mutants are suppressed by the nonpathologic virus? If such a mechanism were to be demonstrated it may be possible to produce an HIV-1 with a Nef
that downregulates CD3 and therefore a dominant, non-pathogenic virus.

A distinctly different explanation of non-pathogenicity is that the sooty mangabey itself has a special mechanism for suppressing the progression to AIDS not the virus [121]. This would explain the fact that rhesus macaques develop AIDS when directly infected with blood from an infected sooty mangabey, but infection of virus-free sooty mangabeys does not [119]. In other words, rhesus macaques die even though the infecting SIV SM’s Nef is downregulating CD3. An additional example of SIV SM being pathogenic when a species barrier is crossed is SIV SM causing AIDS in a black mangabey [122]. Two additional lineages of SIV including African green monkey (SIV AGM) and sun-tailed monkey (SIV SUN) cause AIDS in pig-tailed macaques [123,124], but not that from Sykes’ monkey (SIV SYK) [125]. Like Nef from SIV SM, the Nefs from SIV AGM, SIV SUN, and SIV SYK all downregulate CD3 [22,126]. It should be further noted that the non-pathogenicity of SIV SM is not absolute in sooty mangabeys. Ling et al. have reported a 21 year old sooty mangabey which developed AIDS [121]. These investigators suggest that the evolutionary adaptation to SIV SM is one of delayed progression beyond the usual lifespan of the animal which for sooty mangabeys is under 20 years. In the future it will be important to demonstrate SIV SM and/or SIV AGM specifically defective in CD3 downregulation are significantly pathogenic in their natural hosts.

Schindler et al. have generalized the hypothesis that CD3 downregulation prevents lentivirus pathogenesis by investigating human immunodeficiency virus 2 (HIV-2) [22]. HIV-2 is a zoonotic virus derived from sooty mangabeys [127]. It has reduced pathogenicity relative to HIV-1 overall, but progression to AIDS can occur [128]. Consistent with reduced pathogenicity HIV-2 Nefs do downregulate CD3 [129], but in the cases in which HIV-2 infection has progressed to AIDS one would expect that Nefs derived from these patients would not be functional for CD3 downregulation. This is not the case for Nefs from HIV-2ROD, HIV-2BEN, and HIV-2CBL23 which all came from symptomatic patients and all downregulated CD3 [129]. The pathogenic phenotype of HIV-2 can also be observed in rhesus macaques [130,131]. To demonstrate that CD3 downregulation can block disease progression in humans it will be important to thoroughly investigate the relationship between HIV-2 AIDS and CD3 downregulation.

That a given species may be resistant to its own lentivirus without involvement of CD3 downregulation is clearly demonstrated by the non-pathogenic nature of SIV CPZ. In the case of chimpanzees the downregulation of CD3 cannot serve as a mechanism for non-pathogenicity since SIV CPZ Nef does not downregulate CD3 [22]. Humans lack the ability that chimps have to resist the chimpanzee virus and develop AIDS. Finally, there is evidence that virus with the capacity to downregulate CD3 can not delay HIV-1 pathogenesis. This is suggested by the cases of dual HIV-1/HIV-2 infection. Despite the ability of HIV-2 Nef to downregulate CD3 these patients suffer the greater virulence of HIV-1 [132]. Therefore, it would appear that the non-pathogenic phenotype attributed to Nefs that downregulate CD3 is not dominant over the pathogenic phenotype in humans.

Overall, we conclude that at present there are minimal prospects for therapeutic insights resulting from the attribution of HIV-1 pathogenicity to the inability of HIV-1 Nef to downregulate CD3. If this proposal is to be developed further, it will be necessary to molecularly define the structural correlates of CD3 downregulation. Then the determination could be made if HIV-1 with a Nef modified to downregulate CD3 has reduced pathogenesis in the humanized mouse model [133]. Alternatively, the pathogenesis of HIV-2s with and without the ability to downregulate CD3 could be evaluated for virulence in humanized mice.

Attenuated vaccines

The mechanism of adaptation to SIV SM by sooty mangabeys suggested by Ling et al. [121] is analogous to the approach taken for highly active antiretroviral therapy. Ideally, SIV SM and HIV-1 infections are restrained to be sufficiently chronic and long-lasting that progression to AIDS fails to occur in the life time of the host. This approach is decidedly less desirable than an effective vaccine. Unfortunately, standard vaccine approaches are proven failures. The viral Env has evolved to a highly complex structure that in its native form resists antibody recognition [134]. Broadly neutralizing antibodies (BNAB) do develop during HIV-1 disease that recognize highly conserved epitopes in Env [10], but Env escape variants develop [135,136]. The best hope for preventing HIV-1 infection would be if an attenuated vaccine were to yield BNAB prior to infection. This would allow the immune system to attack the virus early in infection when it is most vulnerable. One positive aspect of the study of the SBBC is that the slow progressors D36, C54, and C98 were able to produce BNAB [15,26]. Unfortunately, these are the three patients that demonstrated disease progression. The LTNPs C49, C64, and C135 had little or no BNAB activity in their blood. So we don’t know if C49, C64, or C135 were “immunized” against HIV-1. Therefore, despite massive efforts to understand the “natural immunization” of SBBC patients with nef-deleted virus there is little evidence that this type of attenuated virus can be effectively or even safely employed.
The progression of D36, C54, and C98, and the failure of C49, C64, and C135 to maintain BNABs have been rationalized by the threshold hypothesis [132]. The divergent patient responses to being exposed to an attenuated virus result from multi-factorial host-virus dynamics. A seroconversion threshold may be reached by a certain level of viral replication, but vaccine protection fails to develop. Conversely, the vaccine threshold may be surpassed resulting in disease development if viral replication is too active. Thus, development of an attenuated virus that can with virtual certainty yield the correct level of replication for non-pathogenicity but still induce a significant and long-lived immune response in the majority of recipients may not be possible. In 1994 the World Health Organization emphasized the importance of determining the optimal combination of genes that could be deleted to ensure safety of an infectious attenuated HIV-1 vaccine [137].

The complexity of defining such a combination of genes is indicated by the report of Churchill et al. [138] that further characterized the two SBBC cohort members that progressed to the point of HAART treatment. The virus from slow progressor, D36, was partially defective in rev, but slow progressor C98 had a fully functional rev. Therefore, the a second defective gene in addition to nef may not reliably further attenuate the virus. As knowledge of the functions of Vif and other HIV-1 accessory proteins grows superior schemes involving combinations of inactivated genes may become apparent for attenuating HIV-1.

Conclusion

Anti-HIV-1 drugs rapidly become ineffective unless administered in multi-drug combinations. More drugs attacking multiple aspects of the viral replication cycle and especially transmission are needed to treat and prevent HIV-1 infection. The enzymatic activities reverse transcriptase and protease have been attacked by anti-virals, but developing drugs that target novel interactions between viral and host cell proteins will be more difficult. Nonetheless, given the relentless nature of HIV-1 all reasonable possibilities should be considered. For Nef the downregulation of CD4 appears to be the most promising function to disrupt by an anti-viral. Evidence in hand indicates this approach has the distinct potential to blunt HIV-1 pathogenesis. MHC-I downregulation is more problematic despite a novel Nef/host cell protein-target interaction because blocking this function may not significantly impact pathogenesis. However, an anti-Nef drug targeting MHC-I downregulation may enhance the ability of a CTL-directed therapeutic vaccine. Pak2 activation may be particularly difficult to target and enhancement of virion infectivity is insufficiently understood to even know what to target.

In a recent development Betzi et al. have identified drug-like compounds (D1 and DCL27) that interact with the SH3 binding domain of Nef [18]. D1 has been observed to interfere with the binding of Hck to Nef, weakly inhibit MHC-I downregulation, but have no effect on CD4 down-regulation. The effect of D1 on MHC-I downregulation may be the result of the proximity of the SH3 binding domain PQVPLR to P78 which is crucial for formation of the ternary complex formed between Nef, the cytoplasmic tail of MHC-I, and AP-1 [43, 45]. Whether D1 binds to cellular SH3 binding domains remains to be determined. Like progress against HIV-1/AIDS, progress in understanding Nef, has been tedious and difficult, but there is no option to continuing to acquire more knowledge about the virus and its proteins.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

Both authors contributed to the writing and editing of the manuscript.

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References

1. Korber B, Gaschen B, Yusim K, Thakallapally R, Kesmir C, Detours V: Evolutionary and immunological implications of contemporary HIV-1 variation. Br Med Bull 2001, 58:19-42.
2. Wain-Hobson S: AIDS. Virological mayhem. Nature 1995, 373:102.
3. Klasse PJ, Shattock RJ, Moore JP: Which topical microbicides for blocking HIV-1 transmission will work in the real world? PLoS Med 2006, 3:e351.
4. Klasse PJ, Shattock R, Moore JP: Antiretroviral drug-based microbicides to prevent HIV-1 sexual transmission. Annu Rev Med 2008, 59:455-471.
5. Sekaly RP: The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development? J Exp Med 2008, 205:7-12.
6. Watkins DI, Burton DR, Kallas EG, Moore JP, Koff WC: Nonhuman primate models and the failure of the Merck HIV-1 vaccine in humans. Nat Med 2008, 14:617-621.
7. Peisutthum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griesven F, Hu D, Tapper JW, Choppaika K: Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. J Infect Dis 2006, 194:1661-1671.
8. Cohen J: AIDS research. Did Merck’s failed HIV vaccine cause harm? Science 2007, 318:1048-1049.
9. Johnson WE, Desrosiers RC: Viral persistence: HIV’s strategies of immune system evasion. Annu Rev Med 2002, 53:499-518.
10. Burton DR, Stanfield RL, Wilson IA: Antibody vs. HIV in a clash of evolutionary titans. Proc Natl Acad Sci USA 2005, 102:14943-14948.
11. Deacon NJ, Tsukin A, Solomon A, Smith K, Ludford-Menting M, Hooker DJ, McPhee DA, Greenway AL, Ellett A, Chatfield C, et al.: Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. Science 1995, 270:988-991.
12. Kirchhoff F, Greenough TC, Brettler DB, Sullivan JL, Desrosiers RC: Brief report: absence of intact nef sequences in a long-term
survivor with nonprogressive HIV-1 infection. N Engl J Med 1995, 332:228-232.

13. Umedo M, Saito TM, Nishizawa M, Sudo K, Iwamuro S, Okabe T, Takeye Y, Imai M: Identification of attenuated variants of HIV-1 circulating recombinant form 01_AE that are associated with slow disease progression due to gross genetic alterations in the neflong terminal repeat sequences. J Infect Dis 2005, 192:56-61.

14. Salvi R, Garbuglia AR, Di Caro A, Pulciani S, Montella F, Benedetto A: Grossly defective nef gene sequences in a human immunodeficiency virus type 1-seropositive long-term nonprogressor. J Virol 1998, 72:3646-3657.

15. Gorry PR, McPhee DA, Verity E, Dyer WB, Wesselingh SL, Learmont J, Sullivan JS, Roche M, Zaunders JJ, Gabuzda D, et al: Pathogenicity and immunogenicity of attenuated, nef-deleted HIV-1 strains in vivo. Retrovirology 2007, 4:66.

16. Coleman SH, Day JR, Guatelli JC: The HIV-1 Nef protein as a target for antiretroviral therapy. Expert Opin Ther Targets 2001, 5:1-22.

17. Greene WC: The brightening future of HIV therapeutics. Nat Immunol 2004, 5:867-871.

18. Betts S, Restout A, Opi S, Arolot ST, Parrot I, Guerlequin F, Morelli M, X Collet,P. Nef-Nef protein interaction inhibition (2P2I) combining high throughput and virtual screening: Application to the HIV-1 Nef protein. Proc Natl Acad Sci USA 2007, 104:19256-19261.

19. Foster JL, Garcia JV: Role of Nef in HIV-1 replication and pathogenesis. Adv Pharmacol 2007, 55:389-409.

20. Luo T, Garcia JV: The association of Nef with a cellular serine/threonine kinase and its enhancement of infectivity are virus isolate dependent. J Virol 1996, 70:6493-6496.

21. Foster JL, Molina RP, Luo T, Arora VK, Huang Y, Ho DD, Garcia JV: Genetic and functional diversity of human immunodeficiency virus type 1 subtype B Nef primary isolates. J Virol 2001, 75:1672-1680.

22. Schindler M, Munch J, Kutsch O, Li H, Santiago ML, Bibollet-Ruche F, Muller-Trutzsch MC, Novembre F, Peeters M, Courgnaud V, et al: Nef-mediated suppression of T cell activation was lost in a lentiviral lineage that gave rise to HIV-1. Cell 2006, 125:1055-1067.

23. Kestler HW 3rd, Ringler DJ, Mori K, Panicali DL, Sehgal PK, Daniel HD, Howe RS: Importance of the nef gene for maintenance of high virus loads and for development of AIDS. Cell 1991, 65:651-662.

24. Learmont JC, Geczy AF, Mills J, Ashton LJ, Raynes-Greenow CH, Garri NJ, Dyer WB, McIntyre L, Oelrichs RB, Rhodes DI, et al: Immuno logical and virologic status after 14 to 18 years of infection with an attenuated strain of HIV-1: A report from the Sydney Blood Bank Cohort. N Engl J Med 1999, 340:1715-1722.

25. Ward JW, Bush TJ, Perkins HA, Lieb AE, Allen JR, Goldfinger D, Samsom JP, Pekopwicz SH, Fernando LP, Holland PV, et al: The natural history of nef-mediated suppression of T cell activation was lost in a lentiviral lineage that gave rise to HIV-1. J Virol 2001, 75:1672-1680.

26. Verity EE, Zotos D, Wilson K, Chatfield C, Lawson VA, Dwyer DE, Cunningham A, Learmont J, Dyer W, Sullivan J, et al: Viral phenotypes and antibody responses in long-term survivors infected with attenuated human immunodeficiency virus type 1 containing deletions in the nef and long terminal repeat regions. J Virol 2007, 81:9268-9278.

27. Churchill MJ, Rhodes DI, Learmont JC, Sullivan JS, Wesselingh SL, Coombe CM, Oelrichs R, Gorry PR: Longitudinal analysis of human immunodeficiency virus type 1 nef-long terminal repeat sequences in a cohort of long-term survivors infected from a single source. J Virol 2006, 80:1047-1052.

28. Bhat N, Learmont JC, Dyer WB, Dearden NJ, Zaunders JI, Saksena N, Cunningham AL, Mills J, Sullivan JS: An examination of signs of disease progression in survivors of the Sydney Blood Bank Cohort (SBBBC). J Clin Virol 2001, 22:263-270.

29. Greenough TC, Brettler DB, Kirchhoff F, Alexander L, Desrosiers RC, O'Brien SJ, Somasundaran M, Luzuriaga K, Sullivan JL: Long-term nonprogressive infection with human immunodeficiency virus type 1 in a hemophilia cohort. J Infect Dis 1999, 180:1790-1802.

30. Greenough TC, Sullivan JL, Desrosiers RC: Declining CD4 T-cell counts in a person infected with nef-deleted HIV-1. N Engl J Med 1999, 340:236-239.

31. Calugi G, Montella F, Favalli C, Benedetto A: Entire genome of a strain of human immunodeficiency virus type 1 with a deletion of nef that was recovered 20 years after primary infection: large pool of proviruses with deletions of env. J Virol 2006, 80:11892-11896.

32. Oelrichs R, Tsykin A, Rhodes D, Solomon A, Ellett A, McPhee D, Deacon N: Genomic sequence of HIV type 1 from four members of the Sydney Blood Bank Cohort of long-term nonprogressors. AIDS Res Hum Retroviruses 1998, 14:811-814.

33. O'Neill E, Kuo LS, Krisko JF, Tomchick DR, Garcia JV, Foster JL: Dynamic evolution of the human immunodeficiency virus type 1 pathogenic factor, Nef. J Virol 2006, 80:1311-1320.

34. Geyer M, Fackler OT, Peterlin BM: Structure–function relationships in HIV-1 Nef. EMBO Rep 2001, 2:580-585.

35. Anderson SJ, Lenburg M, Landau NR, Garcia JV: The cytoplasmic domain of Nef is sufficient for its down-regulation from the cell surface by human immunodeficiency virus type 1. J Virol 1994, 68:3093-3101.

36. Garcia JV, Miller AD: Serine phosphorylation-independent downregulation of cell-surface CD4 by nef. Nature 1991, 350:508-511.

37. Lunsdaine CA, Tobiume M, Zhou J, Unutmaz D, Aiken C: Nef-mediated downregulation of CD4 enhances human immunodeficiency virus type 1 replication in primary T lymphocytes. J Virol 2002, 76:4625-4633.

38. Mangsarsian A, Foti M, Aiken C, Chin D, Carpenter JL, Trono D: The HIV-1 Nef protein acts as a connector with sorting pathways in the Golgi and at the plasma membrane. Immunity 1997, 6:67-77.

39. Atkins KM, Thomas L, Youker RT, Harriff MJ, Pissani F, You H, Thomas M: HIV-1 Nef BINDs PACS-2 to Assemble a Multikinase Cascade That Triggers Major Histocompatibility Complex Class I (MHC-I) Down-regulation: ANALYSIS USING SHORT INTERFERING RNA AND KNOCK-OUT MICE. J Biol Chem 2008, 283:11772-11784.

40. Greenberg ME, Iafraje AT, Skowronski J: The SH3 domain-binding surface and an acidic motif in HIV-1 Nef regulate trafficking of class I MHC complexes. Embo J 1998, 17:2777-2789.

41. Noviello CM, Benichou S, Guatelli JC: Cooperative binding of the class I major histocompatibility complex cytoplasmic domain and human immunodeficiency virus type 1 Nef to the endosomal AP-1 complex via its mu subunit. J Virol 2008, 82:1249-1258.

42. Schwartz O, Marechal V, Le Gall S, Lemonnier F, Heard JM: Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein. Nat Med 1996, 2:336-342.

43. Wondetich ER, Williams M, Collins KJ: The tyrosine binding pocket in the adaptor protein I (AP-1) mu subunit is necessary for Nef to recruit AP-1 to the major histocompatibility complex class I cytoplasmic tail. J Biol Chem 2008, 283:3101-3102.

44. Wei BL, Arora VK, Raney A, Kuo LS, Xiao GH, O'Neill E, Testa JR, Foster JL, Garcia JV: Activation of p21-activated kinase 2 by human immunodeficiency virus type 1 Nef induces merlin phosphorylation. J Virol 2005, 79:14976-14980.

45. Arold ST, Baur AS: Nef-mediated down-regulation of cell-surface CD4 by nef. Nature 1991, 350:508-511.

46. Wonderlich ER, Williams M, Collins KL: The tyrosine binding pocket in the adaptor protein 1 (AP-1) mu subunit is necessary for Nef to recruit AP-1 to the major histocompatibility complex class I cytoplasmic tail. J Biol Chem 2008, 283:3101-3102.

47. Wei BL, Arora VK, Raney A, Kuo LS, Xiao GH, O'Neill E, Testa JR, Foster JL, Garcia JV: Activation of p21-activated kinase 2 by human immunodeficiency virus type 1 Nef induces merlin phosphorylation. J Virol 2005, 79:14976-14980.

48. Arold ST, Baur AS: Nef triggers a transcriptional program in T cells, including single-T cell activation and inducing HIV virulence mediators. Immunity 2001, 14:763-777.

49. Renkema GH, Manninen A, Mann DA, Harris M, Saksela K: Identification of the Nef-asso ciated kinase as p21-activated kinase 2.Curr Biol 1999, 9:1407-1410.

50. Arora VK, Molina RP, Foster JL, Blakemore JL, Chernoff J, Frederick sen BL, Garcia JV: Lentivirus Nef specifically activates Pak2. J Virol 2000, 74:11081-11087.
The human immunodeficiency virus-1 nef gene product: a stimulus-dependent manner.

HIV-1 Nef increases T cell activation in a stimulus-dependent manner. Proc Natl Acad Sci USA 1999, 96:8167-8172.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.

Selective transcription and modulation of cells. Proc Natl Acad Sci USA 1998, 95:1229-1234.
90. Blagoveshchenskaia AD, Thomas L, Feliciani SG, Hung CH, Thomas G: HIV-1 Nef downregulates MHC-I by a PACS-1- and PI3K-regulated ARF6-dependent AGO2 complex, Currillin 2004, 11:183-192.

91. Roeth JF, Collins KL: Human immunodeficiency virus type 1 Nef: adapting to intracellular trafficking pathways. Microbiol Mol Biol Rev 2006, 70:548-563.

92. Walker BD, Chakrabarti S, Moss B, Paradis TJ, Flynn T, Durno AG, Blumberg RS, Kaplan JC, Hirsch MS, Schooley RT: HIV-specific cytotoxic T lymphocytes in seropositive individuals. Nature 1987, 328:345-348.

93. Letvin NL, Walker BD: Immunopathogenesis and immunotherapy in AIDS virus infections. Nat Med 2003, 9:861-866.

94. Brumme ZL, Brumme CJ, Heckerman D, Korber BT, Daniels M, Carls- son J, Kadie C, Bhattacharya T, Chiu C, Stenger J, et al.: Evidence of differential HLA class I-mediated viral evolution in functional and accessory/regulatory genes of HIV-1. PLoS Pathog 2007, 3:e94.

95. Renkema CM, Daniels MG, Carlson JM, Kadie C, Crawford H, Pren- dergast A, Matthews P, Payne R, Rolland M, Raugi DN, et al.: HLA class I-driven evolution of human immunodeficiency virus type 1 subtype C proteome: immune escape and viral load. J Virol 2008, 82:6434-6446.

96. Giorgi JV, Huot LJ, McKeating JA, Johnson TD, Owens B, Jacobson LP, Shih R, Lewis J, Wiley DJ, Phair JP, et al.: Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. J Infect Dis 1999, 179:589-670.

97. Souas AE, Carneiro J, Meier-Schellersheim M, Grossman Z, Victorio RM: CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indi-rectly to the viral load. J Immunol 2002, 169:3400-3408.

98. Ye H, Choi H, Poe J, Smithgall TE: Oligomerization is required for HIV-1 Nef-induced activation of the Src family protein-tyrosine kinase, Hck. Biochemistry 2004, 43:15775-15784.

99. Renkema GH, Manninen A, Saksela K: Human immunodeficiency virus type 1 Nef selectively associates with a catalytically active form of p21-activated kinase 2 in a p21-GTPase-dependent pathway. J Biol Chem 2001, 276:21542-21552.

100. Renkema GH, Manninen A, Saksela K, Benichou S, Guatelli JC: Modulation of cellular protein-tyrosine kinase, Hck. J Virol 2006, 80:14304-14305.

101. Kaur A, Grant RM, Means RE, McClure H, Feinberg MB, Staprans SI: Divergent host responses during primary simian immunodeficiency virus SIVmac239 infection in sooty mangabeys and rhesus macaques. J Virol 2005, 79:5705-5712.

102. Silvestri G, Fedanov A, Germon S, Kozyz N, Kaiser WJ, Garber DA, McClure M, Feinberg MB, Staprans SI: Divergent host responses during primary simian immunodeficiency virus SIVsm infection of natural sooty mangabey and nonnatural rhesus macaque hosts. J Virol 2005, 79:4043-4054.

103. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

104. Moorel I, LaFever-Bennett M, Sichieri F, Huse M, Lee CH, Kurjian J, Miller WT: Activation of the Src-family tyrosine kinase Hck by SH3 domain displacement. Nature 1997, 385:650-653.

105. Briggs SD, Schultz B, Jacque JM, Swingler S, Stevenson M, Smithgall TE: HIV-1 Nef promotes survival of myeloid cells by a Stat3-dependent pathway. J Biol Chem 2001, 276:25605-25611.

106. Breuer S, Gerlach H, Kolaric B, Urbanke C, Opitz N, Geyer M: Biochemical indication for myristoylation-dependent conforma- tional changes in HIV-1 Nef. Biochemistry 2006, 45:2329-2349.

107. Qi M, Aiken C: Selective restriction of Nef-defective human immunodeficiency virus type 1 by a proteasome-dependent mechanism. J Virol 2007, 81:1534-1536.

108. Fackler OT, Luo W, Geyer M, Alberts AS, Peterlin BM: HIV-1 Nef inhibits the early/recovering endosomal compartment correlates with enhancement of HIV-1 infectivity. J Biol Chem 2008, 283:5032-5044.

109. Szilagyi M, Rajan D, Specht A, Ritter C, Pulkkinen K, Saksela K, Briggs SD, Shairkey M, Stevenson M, Smithgall TE: HIV-1 Nef enhances HIV-1 infectivity in association with the virus assembly complex. Virology 2008, 373:287-297.

110. Qi M, Aiken C: Interactions between Nef and AIP1 proliferate multi-vesicular bodies and facilitate egress of HIV-1. Retrovirology 2006, 3:33.

111. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

112. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

113. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

114. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

115. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

116. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

117. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

118. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

119. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

120. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

121. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.

122. Briggs SD, Shairkey M, Stevenson M, Smithgall TE: SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 1997, 272:17999-17992.
associated virus type 2 in AIDS and AIDS-related complex. Clinical and virological features in four patients. Lancet 1987, 1:128-132.

129. Munch J, Schindler M, Wildum S, Rucker E, Baier N, Knoop V, Novembre FJ, Kirchhoff F: Primary sooty mangabey simian immunodeficiency virus and human immunodeficiency virus type 2 nef alleles modulate cell surface expression of various human receptors and enhance viral infectivity and replication. J Virol 2005, 79(10):5471-5460.

130. Stahl-Hennig C, Herchenroder O, Nick S, Evers M, Stille-Siegener M, Jentsch KD, Kirchhoff F, Tolle T, Gatesman TJ, Luke W, et al.: Experimental infection of macaques with HIV-2ben, a novel HIV-2 isolate. AIDS 1990, 4:611-617.

131. Dormont D, Livartowski J, Chamaret S, Guetard D, Henin D, Levagueseres R, Moortelle PF van de, Larke B, Gourmelon P, Vazeux R, et al.: HIV-2 in rhesus monkeys: serological, virological and clinical results. Intervirology 1989, 30(Suppl 1):59-65.

132. Whitney JB, Ruprecht RM: Live attenuated HIV vaccines: pitfalls and prospects. Curr Opin Infect Dis 2004, 17:17-26.

133. Melkus MW, Estes JD, Padgett-Thomas A, Gatin J, Denton PW, Othieno FA, Wege AK, Haase AT, Garcia JV: Humanized mice model specific adaptive and innate immune responses to EBV and TSST-1. Nat Med 2006, 12:1316-1322.

134. Wyatt R, Sodroski J: The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science 1998, 280:1884-1888.

135. Richman DD, Wrin T, Little SJ, Petropoulos CJ: Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc Natl Acad Sci USA 2003, 100:4144-4149.

136. Frost SD, Wrin T, Smith DM, Kosakovsky Pond SL, Liu Y, Paxinos E, Chappay C, Galovich J, Beauchaine J, Petropoulos CJ, et al.: Neutralizing antibody responses drive the evolution of human immunodeficiency virus type 1 envelope during recent HIV infection. Proc Natl Acad Sci USA 2005, 102:18514-18519.

137. Feasibility of developing live attenuated HIV vaccines: conclusions and recommendations. World Health Organization Working Group. AIDS Res Hum Retroviruses 1994, 10:221-222.

138. Churchill MJ, Chiavaroli L, Wesselingh SL, Gorry PR: Persistence of attenuated HIV-1 rev alleles in an epidemiologically linked cohort of long-term survivors infected with nef-deleted virus. Retrovirology 2007, 4:43.