The Role of HIV-1 Nef in Progression, infectivity and replication to AIDS and MHC-I Expression, MHC Class II down modulation as potential target for broad neutralizing antibody vaccine development (Part Ten)

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Abstract

HIV-1 Nef is one of the accessory proteins synthesized in the early stage of HIV-1 reproduction (1). The protein interacts directly with the signal transduction protein of the host T cell and works effectively on AIDS infection or on long term survival of the infected cells or induces apoptosis of non-infected cells.

Recent experimental evidence suggests that Nef might promote T cell activation. Transgenic mice (Tg) expressing HIV-1 nef in CD41 T cells and in cells of the macrophage dendritic lineages developed several AIDS-like pathologies.

In vivo studies or those in primary cell types susceptible to HIV infection have demonstrated that the accessory gene products can dramatically alter the course and severity of viral infection, replication and disease progression.

In vitro, replacement of the codon for threonine 15 by a codon for alanine abolishes phosphorylation by protein kinase C but does not affect the second phosphorylation.

Several reports have suggested that the nef protein is a transcriptional silencer because of its capacity to down regulate viral expression.

In this article, I discuss the role of HIV-1 Nef in Progression to AIDS, Effect of Nef on virion infectivity and replication, General Properties of HIV-1 and SIV Nef Proteins, Nef Biological

Functions, Nef-Mediated Disruption of MHC-I Expression, MHC Class II down modulation and Nef Clinical Significant and Vaccine

Key Words: HIV-1 Nef , CD4, MHC-I, MHC Class II, Apoptosis and Vaccine

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1. Introduction

Nef protein is a 205 aa, N-terminally myristoylated phosphoprotein that has been demonstrated to play a critical role in virus replication and pathogenicity (1).

Besides the three prototypical retroviral proteins (Gag, Pol and Env) and two regulatory proteins (Tat, Rev) that are essential for viral replication, HIV (and simian immunodeficiency virus, SIV) also encodes four so-called accessory proteins called Nef, Vif, Vpr and Vpu (2).

Nef functions can be classified into three categories: (i) effects on protein trafficking, specifically downmodulation of cell-surface molecules such as CD4 and major histocompatibility complex class I; (ii) modulation of signal-transduction pathways such as those emanating from the T-cell receptor; and (iii) enhancement of virus infectivity. Intriguingly, in both virus-infected cells and

cells transfected with Nef expression constructs, a significant proportion of the protein is also found in the cytosol (1),(3),(4),(5).

Because of its capacity to down-regulate viral expression, the nef protein has been classified among the regulatory proteins that control HIV expression, possibly acting like a signal transducing protein (6),(7).

Subcellular localization studies, together with protein–protein interaction data, support a model whereby Nef accelerates the internalization of cell-surface molecules (e.g. CD4) by interacting with the plasma membrane and then recruiting the cellular endocytic machinery to its target protein (8).

Several biological effects of Nef have been identified, including the down-regulation of CD4 and MHC-1, the stimulation of virion infectivity, and the alteration of T cell activation pathways (9),(10). In Jurkat human T lymphoid cells, the surface expression of a CD8-Nef chimera resulted in activation and death by apoptosis whereas its intra-cytoplasmic accumulation led to a state of apparent anergy (11). In an IL-2-dependent rhesus-monkey T lymphoid cell line infected with herpes virus saimiri, nef-positive simian immunodeficiency virus could induce IL-2 production (12). Nef can also bind the T cell-specific Lck (13),(14), and frees Lck from CD4 upon triggering CD4 endocytosis (15),(16).

The present study aimed at HIV-1 Nef in Progression, infectivity and replication to AIDS and MHC-I Expression, MHC Class II down modulation and neutralizing vaccine development

2. The Role of HIV-1 Nef in Progression to AIDS

The clinical manifestations of AIDS are caused by the decline of circulating CD4 T cells which in turn leads to increased susceptibility to opportunistic infections. When the viral load, or number of viral genomes present in the blood, is monitored over the course of HIV infection, a classic disease progression profile emerges: shortly following an exposure, the viral load rises sharply in the blood, until the host acquires HIV-specific cytotoxic T lymphocytes (CTLs) and anti-HIV antibodies; the viral load then settles out to a low-level equilibrium value, termed the viral set point. The relative value of the set point correlates with the rate of progression to AIDS (i.e., a high viral set point typically results in a rapid disease course, and vice versa) (17). Thus, knowledge of the interplay between cellular and viral factors involved in the maintenance of the set point is critical to the understanding of disease progression. The HIV accessory protein Nef has been extensively studied and appears to be a key determinant for viral pathogenesis. One striking example of the pathogenic potential of Nef is a cohort infected with an aberrant HIV strain that contained a large deletion in the *nef* open reading frame. These individuals, designated long-term nonprogressors, have not displayed the typical clinical manifestations of AIDS (18), although after an extended time (14 to 18 years) some have shown some reduction in CD4 counts, consistent with the effects of HIV disease (19),(20). In addition, rhesus macaques infected with an engineered strain of simian immunodeficiency virus (SIV) that lacked a functional Nef protein (SIV_Nef) also did not progress to clinical disease in a timely fashion (21). In fact, SIV_Nef strains have been proposed as candidates for vaccination trials with live attenuated vaccine in the simian model system. While this virus does provide some immune protection against challenge with wild-type virus (22), it can also sometimes cause AIDS itself,

particularly in neonatal macaques (23),(24). Thus, while Nef significantly enhances the ability of HIV to induce AIDS, other HIV factors clearly contribute to the development of disease.

3. Effect of Nef on virion infectivity and replication

The *nef* gene of HIV is critical for pathogenesis and development of AIDS in humans as well as in animal models. Using the Hela-CD4-LTR-□- galactosidase indicator cell line, *nef*+ HIV-1 was found to productively infect 5 to 20-fold more cells than equal amounts of *nef*-defective HIV-1 (25). The infectivity of *nef* defective HIV-1 can be rescued by expressing Nef in *trans* in the virus-producing cell.

The replication rates of *nef*+ and *nef*-defective HIV-1 clones in activated primary blood lymphocytes (PBL) indicated that Nef directly promotes HIV-1 replication by enhancing the infectivity of virions (26). Viruses produced from proviral DNAs mutated in *nef* are 4 to 40 times less infectious than the wild type virus in single-round infection assays. The Nef effect is dependent on the association of this protein with the plasma membrane and is determined at the stage of virus particle formation (27).

The inclusion of Nef in the virion may facilitate the incorporation of Nef-associated cellular kinases that phosphorylate various substrates, including the viral matrix protein, important for the production of fully infectious viral particles (28). Productive HIV-1 infection is also regulated by the ability of Nef to induce the release of a lymphocyte-stimulating factor by macrophages. This leads to an environment in which Nef promotes viral replication in the host by increasing the pool of substrate lymphocytes without additional stimuli (29). The effects of Nef on lymphocyte signaling also involve transcription factors that are induced in response to signaling from the T cell receptor (TCR). Manninen *et al* 34 described a novel effect of Nef on lymphocyte signaling mediated independently of the TCR, which results in induction of nuclear factor of activated T cells (NFAT), a transcription factor that plays a central role in co-ordinating T cell activation (30). The effect of Nef on T cells is mediated by activation of the calcium/ calcineurin pathway and was synergistic with the Ras pathway in inducing NFAT-dependent gene expression.

Ectopic expression of NFAT in resting CD4+ T lymphocytes induced a permissive state, which despite the lack of evidence of T cell activating effects of NFAT overexpression, supported HIV replication in these cells in the absence of further stimulation. HIV replication in these cells is supported by the overexpression of NFAT target genes IL-2 and FasL, in the absence of further stimulation (31). The NFAT protein has also been shown to activate HIV-1 LTR-directed transcription by interacting with an unusual binding site that overlaps with the NF- κB-responsive element.

The Nef-mediated super induction of IL-2 is a reflection of activation of both NFAT and NF-κB sites; thus this mechanism promotes viral replication and spread. The replication of HIV-1 in vitro is restricted to dividing (activated) T cells (32),(33). and studies detailed above explain how Nef enhances viral replication by T cell activation. Swingler et al38 recently proposed an alternative pathway in which Nef intersects the CD40 ligand signaling pathway in macrophages, the first cell type to be infected by HIV. Physiological stimulation of CD40 as also Nef expression in macrophages promotes the release of soluble CD23 (sCD23) and soluble

intercelluler cell adhesion moleculer (sICAM). These in turn upregulate the expression of costimulatory receptors CD22 and CD58 (by sCD23) and CD80 (by sICAM) on B lymphocytes, leading to increased interaction with their corresponding ligands on T lymphocytes.

4. General Properties of HIV-1 and SIV Nef Proteins

The accessory protein Nef was originally named because it was thought to be a negative factor that inhibited viral replication (34), however, it has become clear that Nef positively affects viral replication and infectivity (35),(36). The HIV-1 and SIV Nefs are small (25- to 34-kDa), myristoylated proteins that reside both in the cytoplasm and in association with the cytosolic face of cellular membranes (37). To date, no enzymatic activity has been directly attributed to the Nef protein; however, extensive studies of Nef biology have revealed several conserved motifs that mediate physical association with cellular factors. Consequently, Nef has been hypothesized to function as a molecular adaptor, altering cellular pathways via multiple protein-protein interactions. Nef is able to modulate diverse cellular functions such as protein trafficking events, signal transduction cascades, and apoptotic pathways. Nef is a relatively extended protein containing a large degree of solvent-exposed surface area with several disordered regions (38). Because of this property, it has been difficult to obtain an accurate three-dimensional structure of the full-length protein. However, the structure of the globular core domain (amino acids 54 to 205) of Nef has been solved using X-ray crystallography (39),(40), and nuclear magnetic resonance (NMR) (39),(41). Additionally, the structure of the N-terminal anchor domain has been solved using NMR spectroscopy, and it appears that this domain adopts a relatively unstructured conformation that becomes partially ordered upon the addition of an N-terminal myristyl group (42). **Geyer and colleagues** used these known structures to assemble a structural prediction of the conformation of the full-length Nef polypeptide (43). This model predicts that the surface of Nef consists of a linear array of potential proteinprotein interaction domains and that this surface is quite flexible. Interestingly, it has been speculated that the overall flexibility of Nef enables the protein to switch between multiple conformations and that the structural organization of Nef may be dictated by its binding partner(s) (44). In addition to HIV-1, HIV-2 and SIV also carry the Nef gene, and while several regions are highly conserved, there are also many distinctions. The core domains of HIV-1 and SIV Nef are relatively well conserved and are predicted to fold into a discrete globular domain. In contrast, the amino and carboxy termini are less conserved, and these regions are thought to contain extended loop sequences. In addition, these sequences are enriched in short, linear signal sequences (i.e., tyrosine-based motifs, dileucine motifs, and diacidic motifs) that are known to be recognized by components of vesicular coats. SIV Nef contains more amino acids (and more recognition motifs) in both of its flexible termini.

5. Nef Biological Functions

The clinical manifestations of AIDS are caused by the decline of circulating CD4+ T cells (to <200 cells/μl blood), which in turn leads to increased susceptibility to opportunistic infections. When the viral load, or number of viral genomes present in the blood, is monitored over the

course of HIV infection, a classic disease progression profile emerges: shortly following an exposure, the viral load rises sharply in the blood, until the host acquires HIV-specific cytotoxic T lymphocytes (CTLs) and anti-HIV antibodies (45).

Nef gene, previously referred to as F, 3' orf, orfB or E'. The nefprotein is a 27K protein which is myristylated, localized mainly in the cytoplasm and partly associated with membranes (46),(47). Because of its capacity to down-regulate viral expression, the nef protein has been classified among the regulatory proteins that control HIV expression, possibly acting like a signal transducing protein (6),(48).

Nef is crucial for disease progression (49),(50). Nef binds to m-adaptin and vacuolar ATPase NBP1, and accelerates CD4 internalization by localizing CD4 to CCPs. Nef may then bind to endosomal b-COP, leading to CD4 degradation (51). (60 x 45 x 60 Å,) The myristoylated N-terminus anchors Nef to the membrane (52). Kinase interaction is mediated through an a-helix and a PPII helix RPQVPLR, in the loosely-packed a:b core (50 x 50 x 30 Å,) (53),(40),(54). A protruding loop containing a dileucine motif binds m-adaptin. Residues 57-58 (indicated, left and right) in turn bind to the CD4 dileucine motif.

Nef is a relatively extended protein containing a large degree of solvent-exposed surface area with several disordered regions (38). Because of this property, it has been difficult to obtain an accurate three-dimensional structure of the full-length protein (54),(39),(40).

Recent results from different laboratories suggest that the nefprotein acts as a specific repressor of HIV transcription (55),(56),(57),(58).

By the use of a vaccinia virus recombinant expressing the nefgene, study recently demonstrated that the nef protein is phosphorylated by protein kinase C (on Thr 15 and another unidentified site), has GTP binding and GTPase activities and is also capable of down-regulating CD4 cell surface expression in vaccinia virus-infected CEM T4 cells (6).

Extensive studies of Nef biology have revealed several conserved motifs that mediate physical association with cellular factors. Consequently, Nef has been hypothesized to function as a molecular adaptor, altering cellular pathways via multiple protein-protein interactions.

HIV-1 Nef contributes to HIV pathogenesis by several mechanisms. Nef promotes viral infection by activating CD4 T lymphocytes, which makes them more susceptible to infec-tion. To accomplish this, Nef alters signal transduction pathways downstream of the T-cell receptor (11),(59). Specifically, Nef has been shown to affect molecules that participate in T-cell receptor signaling, such as Vav (60), p21-activated kinase 2 (61),(62),(63), Rac (157), CDC42 (64), and the DOCK2/ ELMO1 complex (65). Interactions with the T-cell signaling pathway also lead to the upregulation of Fas ligand (FasL) on the cell surface (66), which may protect infected cells by promoting apoptosis of neighboring CTLs.

To prevent FasL (and tumor necrosis factor alpha [NF-]) (67), from causing premature death of the infected cell, Nef may bind to andsuppress the activity of ASK1, a kinase that is responsible

for transducing apoptotic signals from FasL and the TNF receptor (68). In addition, Nef inhibits p53-mediated apoptosis(69), and blocks apoptosis via an association with p21-activated kinase and phosphatidylinositol 3-kinases (PI3-kinases) (70). Nef also protects infected cells from CTLs by reducing the cell surface expression of major histocompatibility complex class I (MHC-I), which is required for CTL recognition.

There is a great deal of evidence demonstrating that Nef promotes infection by enhancing the production of infectious HIV virions (71),(72), by at least two mechanisms. First, Nef reduces the expression of the HIV receptor CD4, which can interfere with viral budding and release (73),(74), and second, Nef increases particle infectivity by another, undefined (but CD4-independent) mechanism (75).

In addition, Nef may play a role in the spread of HIV-1 through its effects on dendritic cells (DCs). This cell type can capture HIV-1 particles through a DC-specific receptor (DCSIGN) and later transmit the virus to target cells without becoming productively infected. There is also separate evidence that in some cases, the DCs can themselves become infected (76),(77). In DCs that have become infected, Nef can upregulate DC-SIGN to promote the efficient spread of HIV infection in cocultures of DCs and T cells (78). In macrophages, Nef induces the production of the CC chemokines macrophage inflammatory protein 1α and 1β and other soluble factors to recruit T cells and facilitate productive transmission of HIV infection (79),(80).

If a foreign antigen– MHC-I complex is detected, several responses in the CTL are triggered (including the release of perforins, granzymes, and proapoptotic factors), which lead to the lysis of the infected cell (81).

HIV-1 Nef reduces the surface expression of MHC-I (82), thus preventing the exposure of viral antigens on the surface of HIV-infected cells. This function allows HIV-infected cells to escape recognition and lysis by anti-HIV CTLs in vitro (83), and there is evidence that the ability of Nef to disrupt MHC-I antigen presentation is very important for viral disease pathogenesis in vivo (84),(85),(86).

Reducing the cell surface expression of MHC-I is beneficial in avoiding CTL recognition; however, MHC-I also provides inhibitory signals for natural killer (NK) cells. Thus, infected cells that lack sufficient surface MHC-I expression may become lysed by NK cells (87).

HIV-1 Nef selectively affects some MHC-I allotypes, while ignoring others. Specifically, Nef preferentially disrupts HLA-A and HLA-B expression (88), but not that of HLA-C and HLA-E(89),(90). The preservation of these molecules on the cell surface may provide the proper inhibitory signal to avoid viral detection by NK cells (89).

This differential modulation of MHC-I expression can be mapped to a tyrosinebased sequence (YSQAASS) in the cytoplasmic domains of HLA-A and HLA-B allotypes (90), that is not present

in HLA-C and HLA-E. As a consequence, Nef may protect the HIV-infected cell from both adaptive and innate cell-mediated immunity.

Because of their capacity to form a complex, co-expression of CD4 and the viral envelope disrupts the trafficking of both proteins. Moreover, the presence of CD4 on the cell membrane reduces the ability of the newly formed particle to properly bud and escape the infected cell and therefore reduces viral infectivity. HIV-1 counteracts this effect with the activities of two viral proteins, Vpu and Nef (73).

Nef does not have much of an effect on the transport of CD4 to the cell surface but that it dramatically decreases the half-life of CD4 that has reached the cell surface. Ultimately, Nef targets CD4 for degradation in an acidic compartment (91),(92),(93).

6. Disruption of CD4 Trafficking by Nef

The CD4 protein is a co-receptor required for HIV infection. However, its continued presence on the surface of an HIV-infected cell after viral entry is problematic for several reasons. Because of their capacity to form a complex, co-expression of CD4 and the viral envelope disrupts the trafficking of both proteins. Moreover, the presence of CD4 on the cell membrane reduces the ability of the newly formed particle to properly bud and escape the infected cell and therefore reduces viral infectivity. HIV-1 counteracts this effect with the activities of two viral proteins, Vpu and Nef (73). There is general agreement, based on work from a number of labs, that Nef does not have much of an effect on the transport of CD4 to the cell surface but that it dramatically decreases the half-life of CD4 that has reached the cell surface. Ultimately, Nef targets CD4 for degradation in an acidic compartment (91),(92),(93).

7. Nef-Mediated Disruption of MHC-I Expression

Immune Evasion Antigen presentation by MHC-I provides a mechanism by which a cell can communicate with the extracellular environment specifically, an infected cell can present foreign antigens to signal the presence of a viral infection. CTLs circulate through the body and survey peptide-loaded MHC-I on the surface of cells via the T-cell receptor. If a foreign antigen–MHC-I complex is detected, several responses in the CTL are triggered (including the release of perforins, granzymes, and proapoptotic factors), which lead to the lysis of the infected cell (81).

As with many pathogens that establish a chronic infection, HIV has established ways to subvert the host immune response. HIV-1 Nef reduces the surface expression of MHC-I (94), thus preventing the exposure of viral antigens on the surface of HIV-infected cells. This function allows HIV-infected cells to escape recognition and lysis by anti-HIV CTLs in vitro (95), and there is evidence that the ability of Nef to disrupt MHC-I antigen presentation is very important for viral disease pathogenesis in vivo (84),(85),(86).

Reducing the cell surface expression of MHC-I is beneficial in avoiding CTL recognition; however, MHC-I also provides inhibitory signals for natural killer (NK) cells. Thus, infected

cells that lack sufficient surface MHC-I expression may become lysed by NK cells (87). To perhaps avoid this problem, HIV-1 Nef selectively affects some MHC-I allo-types, while ignoring others. Specifically, Nef preferentially disrupts HLA-A and HLA-B expression (88), but not that of HLA-C and HLA-E (89),(90). The preservation of these molecules on the cell surface may provide the proper inhibitory signal to avoid viral detection by NK cells (89). This differential modulation of MHC-I expression can be mapped to a tyrosine based sequence (YSQAASS) in the cytoplasmic domains of HLA-A and HLA-B allo-types (90), that is not present in HLA-C and HLA-E. As a consequence, Nef may protect the HIV-infected cell from both adaptive and innate cell-mediated immunity.

8. Class II down modulation

The MHC II protein is expressed in all APCs and present antigenic peptides to CD4+ T cells Further, human macrophages accumulate HIV-1 particles in MHC II compartments (96), and MHC II can be incorporated in the virion (97).. In chronically infected monocytes, MHC II antigen presentation is hampered, and HIV-1 Nef is reported to affect the surface localization of MHC II proteins, reducing presentation of exogenous peptides to CD4+ cells (98),(99),(100).

The Nef protein disrupts MHC II antigen presentation by two distinct mechanisms. These include down regulating surface expression of mature MHC II and up-regulating surface expression of MHC II associated invariant chain (li, CD74). Higher amounts of Nef expression are required for mature MHC II down modulation compared to up-regulation of Ii. Nef-alleles from primary HIV-1 cause surface up-regulation of Ii, a function that is genetically separable from MHC II surface downmodulation (100).

For example, the acidic domain of Nef is involved in down-regulation of MHC II but is dispensable for Ii up-regulation. Mutations in the C-proximal flexible loop consistently abolish the ability of Nef to modulate Ii surface expression but had little effect on down-regulation of MHC II. The dileucine motif LL165/166 and the acidic motif EDE174-176 are critical for Nef-induced down-regulation of CD4 and up regulation of Ii. Mutations in Pro75 and Pro78 block MHC I and MHC II down modulation but have no effect on Ii up-regulation, suggesting that an intact SH3-binding PxxP motif is not required for Ii up-regulation but is required for MHC II modulation (98),(99),(100).

9. Nef and apoptosis

Nef induces the expression of both Fas (CD95) and the Fas ligand (CD95L) in infected cells (101),(102), with CD95L aiding immune evasion by inducing the apoptosis of HIVspecific cytotoxic T-cells (CTLs) (101). The apoptosis signal regulating kinase 1 (ASK1) is a key signaling intermediate in the Fas and TNF-α death signaling pathways and was reported to bind Nef (103). This association is reported to result in the inhibition of ASK1 kinase activity and the downstream induction of c-Jun N-terminal kinase (JNK) and apoptosis (104). The binding of Nef to ASK1 is lost on disruption of its N-terminal myristoylation site as well as by a R106A mutation, which is implicated in the binding of a p21–activated kinase (PAK) family protein (105).

The association of Nef with ASK1 reveals a mechanism by which HIV-1 Nef can alter the intracellular milieu of virally infected host cells by enhancing their resistance to Fas and TNFα mediated apoptosis. The Nef protein also represses death signaling, a pro-apoptotic member of the Bcl-2 protein family whose expression is induced by HIV, and that triggers apoptosis at the level of mitochondria (105). The Nef-mediated activation of PI3K and PAK results in the phosphorylation resulting of Bad in release of the anti-apoptotic Bcl-XL protein from a Bcl-XL complex and enhancement of cell survival and virus production (106). Through its N-terminus (residues 1-57) Nef also interacts with the p53 tumour suppressor protein. This interaction results in the destabilization of p53, thereby decreasing its pro-apoptotic, transcriptional and DNA-binding activities, and protecting HIV-1 infected cells from p53-mediated apoptosis (69). The inhibition of p53 further enhances the anti-apoptotic effects of Nef in the infected cell.

10. Nef Clinical Significant and Vaccine

The expression of Nef early in the viral life cycle ensures T-cell activation and the establishment of a persistent state of infection, two basic attributes of HIV infection. Viral expression of Nef induces numerous changes within the infected cell including the modulation of protein cell surface expression, cytoskeletal remodeling, and signal transduction. Since the activation state of the infected cell plays an important role in the success rate of HIV-1 infection, it is important that resting T-cells be primed to respond to T-cell receptor (TCR) stimuli. HIV-1 Nef lowers the threshold for activation of $CD4^+$ lymphocytes, but is not sufficient to cause activation in the absence of exogenous stimuli (107).

By down regulating cell surface expression of CD4 and Lck, Nef creates a narrow TCR response which likely optimizes HIV-1 viral production and generates a susceptible population of cells to further infect. Nef retargets kinase-active Lck away from the plasma membrane to early and recycling endosomes (RE) as well as the Trans-Golgi network (TGN). RE/TGN associated Lck sub-populations in Nef expressing cells are in the catalytically active conformation and thus signaling competent (108). While TCR signaling takes place at the plasma membrane (PM), activation of the Ras-GTPase takes place in intracellular compartments including the Golgi apparatus. Nef induced enrichment of active Lck in these compartments results in an increase of localized RAS activity and enhanced activation of Erk kinase and the production of Interleukin-2 (IL-2) (109). Since IL-2 is known to activate the growth, proliferation, and differentiation of T-cells to become effector T-cells; this is a self-serving effect that creates a new population of cells which HIV-1 is able to infect. Self-activation of the infected cell by IL-2 also stimulates the cell to become an effector cell and initiate the machinery which HIV-1 relies upon for its own proliferation.

To further evade the host immune response, Nef down-regulates the cell surface and total expression of the negative immune modulator CTLA-4 by targeting the protein for lysosomal degradation. In contrast to CD28 which activates T-cells, CTLA-4 is essentially an "off-switch" which would inhibit the viral production if it were activated.

Nef is also known to phosphorylate and inactivate Bad, a proapoptotic member of the Bcl-2 family thus protecting the infected cells from apoptosis.

Cytoskeletal remodeling is thought to reduce TCR signaling during early infection and is also modulated to some degree by Nef. Actin remodeling is generally modulated by the actin severing factor cofilin. Nef is able to associate with the cellular kinase PAK2 which phosphorylates and inactivates cofilin and interferes with early TCR signaling.

11. Conclusion

The wild-type nef protein is phosphorylated by two distinct protein kinase activities at different sites: a site containing a threonine residue, possibly phosphorylated by protein kinase C, and sites containing serine, phosphorylated by an autophosphorylation process (6),(110). Nef protein plays a central role by down modulating the surface expression of CD4, MHC I, MHC II and CD28 proteins critical for the formation of an immune synapse. Correspondingly, in professional antigen presenting

Cells such as macrophages and dendritic cells that are the first cells to be infected by HIV, Down modulation of surface MHC I and MHC II ensures poor presentation of HIV peptides to TCRs on helper or cytotoxic T cells. In addition, Nef mediated reduction in MHC I expression on the surface of HIV-infected cells helps these cells escape from virus-specific CTLs.

The overall effect of Nef is to reduce T cell help for generating effective virus-specific antibody and cytotoxic responses, as well as reduce the engagement of infected cells and virus-specific CTLs. The Nef protein also promotes the survival of infected cells by inhibiting "outside-in" as well as "inside-in" death signals; in the latter case, p53-independent as well as dependent pathways are invoked.

Besides an active role in promoting viral persistence, Nef initiates a transcriptional programme in T cells similar to that seen in TCR-activated T cells. The Nef protein interacts with a wide range of cellular proteins, many known to perform critical functions in signaling pathways. Structure-function studies on the Nef protein have identified a number of motifs involved in protein protein interactions and favour a model wherein Nef acts as an adaptor to accumulate signaling complexes in the infected cell.

To date, most of the activities of the Nef protein have been attributed to the intracellular expression of the protein or its association with virions. Inside cells, Nef is known to downregulate CD4 and major histocompatibility receptors.(111),(94).

To accomplish these functions, Nef acts as a sort of connector between the receptor and components of the cell's trafficking machinery (88). Nef binds to CD4 by a dileucine-based signal in the CD4 cytoplasmic tail. Nef's N-terminal myristoylation signal directs it to membranes and its site of action,though recent reports implicate basic residues near the N-terminus as also important.(112). The Nef that is secreted from these cells is also in a form that can be sedimented at high centrifugal forces. Using a Nef-GFP expression vector, we also show that Nef vesicles can be visualized and that the vesicles can fuse with target Jurkat cells.

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