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HIV-1 Nef Transfer and Intracellular Signalling in Uninfected Cells

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1. Introduction

Several lymphotropic viruses manipulate host innate immune response to escape immune recognition and improve viral replication and spreading. From this point of view HIV (Human Immunodeficiency Virus-1) represents a paradigmatic example (for review see Peterlin & Trono, 2003). HIV-1 encodes the classical structural and enzymatic factors of all retroviruses codified from the *gag* (group-specific antigen), *pol* (polymerase) and *env* (envelope) genes. In addition it codes two regulatory proteins that are essential for viral replication (i.e., the transcriptional transactivator Tat and the regulator of virion gene expression Rev) and four accessory proteins (i.e., the ill-named 'negative effector' Nef, the viral infectivity factor Vif, the viral protein r Vpr and the viral protein u Vpu). With time has become increasingly clear that the so-called accessory proteins carry out several critical functions for both viral replication and pathogenesis (Malim & Emerman, 2008). In particular, the Nef protein was demonstrated to be an important virulence factor of primate lentiviruses. In fact Nef-defective HIV leads to an attenuated clinical phenotype with reduced viral loads in mouse models, monkeys, and in human disease (Daniel et al., 1992; Deacon et al., 1995; Gulizia et al., 1997; Kestler et al., 1991; Kirchhoff et al., 1995) and *nef* transgenic mice develop an AIDS-like disease (Hanna et al., 1998) confirming that this viral protein is a major determinant of pathogenicity.

Studies on structure and mechanism of action of the protein highlighted its multifunctional properties at cellular and molecular level. The open reading frame encoding Nef is located 3′ of the *env* gene, overlaps the untranslated sequences of the 3′ viral long terminal repeat and is translated from multiply spliced transcripts. Nef is expressed early and most abundantly during the infection cycle together with Tat and Rev and evidences have been reported of possible expression also before integration of the proviral genome (Wu & Marsh, 2001). It acts as a molecular adaptor inside the cell inducing genetically distinguishable, yet highly

conserved, effects via specific protein-protein interaction motifs (Arold & Baur, 2001; Doms & Trono, 2000; Geyer et al., 2001, Foster et al., 2011). In general, Nefs from all primate lentiviruses (*i.e.* HIV-1, HIV-2, and SIV) share multiple activities *in vitro*, but differences between the ability of human and simian immunodeficiency viruses to induce Nef-mediated internalization of the CD3 component of the T-cell receptor complex have been observed and have been correlated with the greater pathogenicity of HIV and the reduction of T-cell activation in simian immunodeficiency virus (SIV) (Schindler et al., 2006). It has been also observed that HIV-1 Nef treatment of uninfected cells in culture causes internalization of the protein in some cell types and/or activates specific intracellular signalling pathways. Interestingly, Nef has been found inside uninfected B cells of lymphoid follicles from infected individuals (Qiao et al., 2006) and recently it has been provided experimental evidencies that it can be transferred to uninfected cells from the infected ones via cellular protrusions and/or exosomes (Lenassi et al., 2010; Muratori et al., 2009; Xu et al., 2009) opening a new road to deepen our insight on the roles of this multifunctional protein. This review will focus on those recent observations trying to provide a unifying reading.

2. Nef: A multifunctional viral adaptor

Nef is a protein of about 200 aminoacids and the different alleles may vary slightly in length. Sequence analysis has identified a number of conserved motifs that are responsible for protein–protein interactions and for specific biological functions. The protein is co-translationally modified by N-terminal myristoylation and is phosphorylated on specific aminoacid residues. Its membrane binding is critical for Nef function on cell signalling and membrane trafficking and requires both the covalently attached myristic acid moiety and a cluster of N-terminal basic residues (Bentham et al., 2006; Gerlach et al., 2010; Szilluweit et al., 2009). Myristoylation appears only a weak membrane-targeting signal, but the N-terminal basic residues, especially the arginine-rich cluster (R^{17} to R^{22}), are needed for the stable association of the viral protein with cellular membranes. Nef appears to be a cytoplasmic protein partially associated with cell membrane and often accumulated in perinuclear regions. Cellular-fractionation assays from transient transfection experiments showed that less than 60% of the protein is localized at membranes, while the remaining portion was found to be cytosolic (Kaminchik et al., 1994). Structurally the 24-29 kDa HIV-1 Nef protein adopts a two-domain structure encompassing a flexible membrane anchor domain (residues 2-61 in Nef_{SF2}) and a folded core domain (residues 62-210). The core domain, again, contains a C-terminal flexible loop of 33 residues (152-184) that is thought to mediate trafficking interactions (Geyer et al., 2001). Arold and Baur speculated that after translation the protein adopts a close conformation where the myristoyl moiety interacts with a hydrophobic region on the core domain, which could explain why the majority of the protein is localized in the cytosol and not attached to membranes (Arold & Baur, 2001). Biochemical indication for myristoylation-dependent conformational changes in HIV-1 Nef has been obtained (Breuer et al., 2006; Dennis et al., 2005). Indeed, Nef is readily soluble in aqueous solution, suggesting the shielding of the lipid moiety within the protein. Protein structures of Nef have been determined for the core domain by NMR spectroscopy and X-ray crystallography (Arold et al., 1997; Grzesiek et al., 1997; Lee et al., 1996) and for the flexible anchor domain by NMR (Geyer et al., 1999), but not yet for the full-length protein due to the low stability and solubility and the high degree of intrinsic flexibility. The protein mediates a multitude of functions, increasing the production and infectivity of viral particles

and inducing alteration of specific cellular signalling and trafficking pathways. It has been also demonstrated that the protein is able to induce the transient translocation to the cell membrane of the Polycomb Group protein Eed, a nuclear transcriptional repressor, leading to a potent stimulation of Tat-dependent HIV transcription (Witte et al., 2004). The cytoplasmic translocation of Eed seems to result in the removal of a block on Tat-mediated HIV transcription essential to promote viral transcription at low concentrations of the transactivator protein found at the very early phase of the infection or reactivation from latency. It has been proposed that Nef adopts different structural conformations inside the cell that allows different localizations and interaction with different partners, realizing the so called "Nef interaction cycle" (Arold & Baur, 2001). In particular, Nef, after translation, could adopt the closed conformation in which its binding sites are mainly hidden (closed conformation). Contact with the cell membrane could then trigger a conformational change via the interaction of the negative charges of the membrane lipid heads with the positive charges in the N-terminus of Nef, thus relieving the interaction between the N-terminus and the core. This conformational change could also expose several motifs capable of binding signalling molecules (signalling conformation), many of which are present in lipid rafts. The association of Nef molecules with the membrane might persist for only a short period of time because of the exposure of the core loop, which would then bind molecules of the cellular endocytotic machinery mediating internalization of Nef together with specific interaction partners.

The best characterized Nef functions are: (a) acceleration of endocytosis and lysosomal degradation of CD4, thereby avoiding both super-infection of infected cells and the interaction of budding virions with CD4 of the infected cells; (b) down-regulation of HLA-A and -B MHC-I molecules thereby protecting infected cells from recognition and lysis by cytotoxic T lymphocytes (CTL); (c) induction of a pre-activation state in CD4+ T cells favouring viral gene expression (Simmons et al., 2001); (d) regulation of apoptosis, promoting apoptosis in bystander uninfected cells meanwhile protecting the infected cells by apoptotic stimuli through more than one mechanism (Das & Jameel, 2005). Nef perturbs the trafficking of many different plasma membrane-associated proteins but how Nef traffics within the endosomal system to reach the perinuclear endosomal region where it is concentrated at steady state is not yet understood (Burtey et al., 2007; Roeth & Collins, 2006). The list of cellular proteins whose transport is affected has continued to grow over the past several years. To date it has been reported to decrease the cell surface expression of MHC-I, MHC-II, CD4, CD28, transferrin and mannose receptors, CD80, CD86, CD8, and CCR5 and to increase the expression of TNF, LIGHT, DC-SIGN, and the invariant chain (Anderson et al., 1993; Chaudhry et al., 2005; Lama & Ware, 2000; Madrid et al., 2005; Schindler et al., 2003; Schwartz et al., 1996; Sol-Foulon et al., 2002; Stove et al., 2005; Stumptner-Cuvelette et al., 2001). The acceleration of endocytosis and lysosomal degradation of the transmembrane glycoprotein CD4 was one of the first discovered actions. To recruit CD4 into the endocytotic pathway, Nef acts as an adaptor between CD4 and components of the clathrin coated pits. In T cells this involves disruption of the CD4-Lck tyrosine kinase complex by Nef and its interaction with adaptor protein (AP) complexes and the regulatory subunit of the vacuolar proton pump v-ATPase. Nef binds also to the beta subunit of COPI coatomers (β-COP) to direct CD4 to a degradation pathway. Surprisingly, Nef uses different domains and mechanism to downregulate MHC-I molecules (Blagoveshchenskaya et al., 2002; Doms & Trono, 2000). Regarding the Nef ability to induce a state of pre-activation in T cells several publications have reported its direct interaction with T Cell Receptor (TCR) ζ chain,

glycolipid enriched membrane microdomains (i.e., GEMs also called lipid "rafts") and proteins of the submembrane TCR environment including the adaptor proteins Vav and LAT, and the kinases Lck, PAK and PKC (Renkema & Saksela, 2000). Mutational analysis suggests that most of the signaling molecules that bind to Nef interact with its core domain, often via the Pro-rich sequence that binds the SH3 domain of Src kinases. Nef displays high (*i.e.* nanomolar) affinity for the SH3 domain of Hck and Lyn, and rather modest (*i.e.* micromolar) affinity for those of Lck, Fyn and Src. *In vivo,* the individual tissue distribution of Src kinases become relevant because Hck is restricted to macrophages, therefore in T cells Nef might interact with lower-affinity targets such as Fyn or Lck. As far as regulation of apoptosis concerns, it appears that Nef causes bystander cell apoptosis in uninfected cells through its induction of FasL expression on the infected $CD4^+$ T cell (Xu et al., 1999; Xu et al., 1997). Conversely, it inhibits apoptosis in virally infected host T cells both through its concomitant suppressive effects on ASK1, a key intermediate in the Fas and TNFα death signaling cascade, and via Akt-indipendent phosphorylation of Bad (Geleziunas et al., 1996; Geleziunas et al., 2001; Wolf et al., 2001). In addition it has been described to inhibit p53-mediated apoptosis binding the tumor suppressor (Greenway et al., 2002).

As a consequence of Nef ability to bind multiple targets it is plausible that depending on Nef subcellular localization and the availability of particular subset of targets (for example Hck versus Lck tyrosine kinase), certain Nef effects dominate over others in a time- and cell type-dependent manner thus differing in infected monocyte/macrophages versus T lymphocytes, DC, astrocytes or microglial cells (see Quaranta et al., 2009 for the different regulatory effects of Nef in immune cells). In macrophages, that are important target of HIV infection and reservoir of the virus, Nef expression induces the release of a set of paracrine factors including a marked increase of CCL2/MIP-1α and CCL4/MIP-1β chemokines. These factors are able to recruit T cells and make them susceptible to HIV replication (Swingler et al., 2003; Swingler et al., 1999). In particular, Nef expression in macrophages induces also the production of the soluble forms of the intercellular adhesion molecule ICAM-1 (sICAM) and of the coactivation molecule CD23 (sCD23). sICAM and sCD23 act on B cells that cooperate with macrophages in inducing T cell recruitment and their permissivity to productive infection *in vitro*. The effects of Nef expression in macrophages mimics those of CD40 Ligand (CD40L) in activating the CD40 signalling cascades suggesting that Nef intersects a macrophages pathway that is regulated by the CD40 receptor and requires nuclear factor kappa B (NF-κB) activation (Swingler et al., 2003; Swingler et al., 1999).

3. Effects induced by extracellular Nef in uninfected cells

Evidences that Nef produced by recombinant DNA technology can induce cell signalling effects when added to cell cultures has been provided (Alessandrini et al., 2000; Brigino et al., 1997; Fujii et al., 1996c; Fujinaga et al., 1995; Huang et al., 2004; James et al., 2004; Lehmann et al., 2006; Okada et al., 1998; Okada et al., 1997; Qiao et al., 2006; Quaranta et al., 1999; Quaranta et al., 2003; Tobiume et al., 2002; Varin et al., 2003). Different Nef alleles have been expressed in *E. coli* and both myristoylated (myr^+) or not myristoylated (myr^-) proteins have been produced (Breuer et al., 2006; Dennis et al., 2005). Myr^+ Nef was obtained via co-trasformation of *E. coli* with expression vectors coding the viral protein and the human N-myristoyl-transferase and supplementing the culture medium with myristic acid. Purified myr^+ and myr^- wild type (wt) Nef proteins showed different oligomerisation properties *in vitro*. Indeed, myristoylated Nef prevails in a monomeric state in solution whereas the

nonlipidated protein forms dimers, trimers, or even oligomers of greater magnitude. When cell signalling effects induced by myr+ and myr- wtNef were compared in cultures of human monocyte-derived macrophages (MDMs), myr+ wt Nef was found to be much more active than the myr- protein (Mangino et al., 2007; Olivetta et al., 2003). Table 1 briefly summarizes the effects induced by cell treatment with Nef. In particular, negative effects on $CD4^+$ T cell survival has been described (Fujii et al., 1996a; b; Fujii et al., 1996c), *e.g.* Nef treatment of $CD4^+$ T lymphocytes in culture induced apoptosis via the interaction with the CXCR4 receptor (Huang et al., 2004; James et al., 2004). On immature dendritic cells (iDCs) exogenous Nef enhances CXCR4 expression and up-regulates MHC-II molecules, possibly favouring their migration and nonspecific $CD4^+$ T cell activation (Quaranta et al., 2002). Exogenous Nef enters iDCs, promoting their functional and morphological differentiation. Specifically, Nef promotes interleukin (IL)-12 release, which closely fits with nuclear factor NF-κB activation, and targets Vav promoting its tyrosine phosphorylation associated with its nucleus-to-cytoplasm redistribution. Nef induces also the rearrangement of actin microfilaments, leading to uropod and ruffle formation and increases the capacity of DCs to form clusters with allogeneic $CD4^+$ T cells, improving immunological synapse formation (Quaranta et al., 2003). In addition to iDCs, Nef is internalized by primary human MDMs and IgD^+ B cells in culture (Alessandrini et al., 2000; Qiao et al., 2006). Myr+ Nef treatment of primary human IgD^+ B cells induced to differentiate in culture by the addition of CD40L, IL-4 and IL-10 inhibits switching to IgG, IgA, and IgE by inducing the negative regulators I-κB-α and SOCS proteins, which block CD40L and cytokine signalling rendering Nef-containing B cell less responsive to $CD4^+$ T cell help (Qiao et al., 2006). Nef treatment of primary human MDMs down-regulated CD4 surface expression, thus reproducing an effect widely observed in cells endogenously expressing the viral protein. In addition, myr+ Nef treatment of MDMs induces the rapid (15-30') activation of IKK/NF-κB, MAPKs (i.e., ERK1/2, JNK and p38) and IRF-3, the main transcriptional regulator of the IFNβ gene expression, thereafter regulating the expression of many cellular transcripts (Federico et al., 2001; Mangino et al., 2007; Olivetta et al., 2003; Percario et al., 2003). The prompt transcriptional reprogramming leads in 2 hours to the synthesis and release of a set of proinflammatory cytokines/chemokines, including TNFα, IL-1β, IL-6, CCL2/MIP-1α and CCL4/MIP-1β, and of IFNβ that, in turn, immediately activate the signal transducers and activators of transcription STAT1, -2 and -3 in autocrine and paracrine manner. A transient STAT1, -2 and -3 tyrosine phosporylation was also observed early after (*i.e.* 8-16 hrs later) *in vitro* infection of 7-day old human MDMs with *nef*-expressing Δ*env*, but not Δ*nef*/Δ*env*, HIV-1 pseutotypes suggesting that intracellular signalling induced in Nef-treated MDMs might also be activated via Nef intracellular expression soon after MDMs infection with HIV-1 or viral reactivation from latency. In an attempt to identify the Nef structural motifs required for the activation of those signaling pathways, MDMs were treated with different myr^+ $recNef_{SF2}$ proteins lacking specific conserved aminoacid residues (Mangino et al., 2007). In particular, the viral protein was modified in the consensus sequence required for myristoylation (mutant $G^2 \rightarrow A$), in the polyproline-rich region (mutant $P^{76}XXP^{79}XR^{81} \rightarrow AXXAXA$), in the domain required for the interaction with CD4 (mutant $C^{59}AWL^{62} \rightarrow AAAA$) or with elements of the endocytic machinery, such as the V1H subunit of the vacuolar-membrane ATPase (mutant $E^{178}D^{179} \rightarrow AA$) or the adaptor protein complex (mutant $L^{168}L^{169} \rightarrow AA$) Finally a Nef protein with a deletion of the first 44 amino acids (ΔN-term) was also used. Overall the experimental results indicated that myristoylation of the protein was required for the activation of the signaling cascades, because $G^2 \rightarrow A$ and ΔN-

term Nef were the only two Nef mutants unable to induce signalling. These data were in agreement with lack of induction of STAT1, STAT2 and STAT3 tyrosine phosphorylation in MDMs 8 to 16 h after infection with VSV-G Δ*env* HIV-1 pseudotypes expressing the $G^2 \rightarrow A$ Nef mutant (Mangino et al., 2007; Percario et al., 2003). As previously mentioned myristoylation of Nef is a weak membrane-targeting signal and N-terminal basic residues, especially an arginine-rich cluster (R^{17} to R^{22}), are needed for the stable association of the viral protein with cellular membranes. Presently, it is not possible to exclude the hypothesis that also these residues are important together with myristoylation for the activation of NF-κB and IRF-3 in MDMs.

Regarding the production of IFNβ we propose that Nef, abundantly expressed early after HIV infection together with Tat and Rev, induces IFNβ at a very early stage of viral infection or soon after reactivation of viral gene expression in latently infected cells, before the appearance of other viral proteins that can inhibit type I IFN production or mechanisms of action to allow the viral release from infected cells. In fact, even if type I IFNs inhibit the release of retroviral particles and their infectivity in cells chronically infected by the simplest retroviruses without inducing relevant reduction of viral proteins synthesis (Peng et al., 2006; Pitha-Rowe & Pitha, 2007), the lentivirus HIV has evolved specific strategies to inhibit IFN-mediated antiretroviral effects at least via Vif and Vpu accessory protein functions. Both these viral proteins are produced later than the early HIV regulatory protein Nef and inhibit the function of APOBEC3 and tetherin, two cellular antiretroviral proteins specifically increased by type I IFN via positive transcriptional regulation (Malim & Emerman, 2008). In addition a recent analysis of antiviral defences in $CD4^+$ cells during HIV-1 infection indicates that viral expression can also direct a global disruption of innate immune signalling through suppression of IRF-3 (Doehle et al., 2009). In particular, a marked depletion of IRF-3, but not IRF-7, was observed in HIV-1-infected cells which supported robust viral replication. Indeed, IRF-3 depletion was dependent on a productive HIV-1 replication cycle and caused the specific disruption of Toll-like and RIG-I-like receptors innate immune signalling. In agreement with these *in vitro* results, IRF-3 levels were found reduced *in vivo* within $CD4^+$ T cells from patients with acute HIV-1 infection, but not from long-term non-progressors (Doehle et al., 2009). A decrease of IRF-3 expression after HIV-1 infection was observed also in THP-1 cells, a human monocyte/macrophage-like cell line, as HIV-1 infection progressed over a 48-h period. The ability of Nef to induce transient IFNβ production in HIV-1 infected cells *in vivo* needs to be investigated. Nef might induce IFNβ production in cells neighbouring the infected ones if the protein is secreted or released after cell lysis or when transferred via cell-to-cell contact to uninfected cells (Peterlin, 2006). This cytokine could exert its effects on bystander cells, such as the plasmacytoid dendritic cells, priming them to IFNα production. Other HIV products (*i.e.*, gp120) induce the production of type I IFNs in MDMs, as well as in PBMCs (Ankel et al., 1994; Capobianchi et al., 1993; Capobianchi et al., 1992; Gessani et al., 1994). Induction of both IFNα and -β as a consequence of *in vitro* infection of MDMs cultures has been reported (Gessani et al., 1994; Szebeni et al., 1991) and a rapid and transient elevations in IFNα has been observed during the cytokine storms that accompany the increase in plasma viremia in acute HIV-1 infection (Stacey et al., 2009). It is interesting to note that in early 1980's one of the first clear-cut HIV isolate was obtained using cultured T lymphocytes, derived from a lymphonode biopsy specimen of a patient with lymphadenopathy, with the help of IL-2 and anti-IFNα serum (Barre-Sinoussi et al., 1983).

Ref.	recNef Alleles	Cell type	Induced Effects
Alessandrini et al., 2000	NL4-3	primary hu MDMs	- CD4 downmodulation - Inhibition of M- and Dual-tropic strains replication
Brigino et al., 1997	LAV	PBMC H9 U937	- IL-10 induction in a calcium/calmodulin dependent manner
Creery et al., 2002		PBMC primary hu monocytes	- CD14 upregulation
Federico et al., 2001 Mangino et al., 2007 Olivetta et al., 2003 Percario et al., 2003	NL4-3 BH10 SF2	primary hu MDMs	-- Activation of NF-kB, MAPKs and IRF-3 signalling pathways - Synthesis of pro-inflammatory cyto- and chemokines including CCL2/MIP-1α, CCL4/MIP-1β, TNFα, IL-1β, IL-6 and IFNβ that induce STAT1, STAT2 and STAT3 phosphorylation in autocrine and paracrine manner - Induction of IRF-1
Fujii et al., 1996 a,b,c	NL4-3	$CD4^+$ T lymphocytes	- Cytotoxic effects on T cells - Inhibition of proliferaton
Fujinaga et al., 1995		MOLT-20-2	- HIV reactivation from latency
Huang et al., 2004	NL4-3	PBMC Jurkat	- Apoptosis induction through Nef-CXCR4 interaction
James et al., 2004	NL4-3	Jurkat H9	- Apoptosis induction
Okada et al., 1997 Okada et al., 1998	ELI III B	JA-4, Balb3T3 EL-4, WEHI	- Fas-independent apoptosis induction
Qiao et al., 2006	BaL	primary B cells	- Suppression of CD40-dependent Ig class switching
Quaranta et al., 1999	ELI	hu monocyte /macrophages	IL-15 synthesis induction
Quaranta et al., 2002 Quaranta et al., 2003	ELI	primary hu Dendritic Cells	- DC maturation - Cytoskeleton rearrangements through Vav/Rac-1 dependent signaling
Tobiume et al., 2002			- HIV activation from latency through Ras/MAPKs signaling
Varin et al.,2003	SIVmac SF2 BH10	U937 U1	- Stimulation of HIV transcription through NF-κB, AP-1 and c-JNK signaling

Table 1. Effects induced by cell treatment with recombinant Nef

Regarding the response of astrocytes to extracellular Nef, treatment of uninfected cells (*i.e.* human glioblastoma/astrocytoma cell line U373MG) with the purified viral proteins Nef induces the synthesis of C3 (Speth et al., 2002). A significant difference in the C3 synthesis in Nef-treated cells was visible after 3 days and maximal effect was reached after 9 days. Besides Nef, also whole HIV virions and gp41 were biologically active in upregulating C3, whereas Tat, gp120, and gp160 were not (Speth et al., 2002). The complement system is of special importance in the brain because the elements of adaptive immunity have only

limited access due to the blood-brain barrier. The complement factor C3 is a central protein of the cascade, and its fragments (C3b, iC3b, C3d, and C3a) affect many cellular processes in the brain, such as activation of signalling pathways and modulation of cytokine synthesis. In general, all complement proteins can be synthesized by various brain cells, including astrocytes, neurons, microglia, and oligodendrocytes, with astrocytes being the most potent complement producers. Although normal synthesis in the brain is low, inflammatory cytokines such as IFNγ and TNFα considerably increase complement production, especially of complement factor C3. Increased levels of C3 and C4 were found in the cerebrospinal fluid of HIV-infected patients with neurological symptoms and signs of central nervous system dysfunction, supporting the hypothesis of an association between complement and HIV-induced neurodegeneration. HIV-induced upregulation of C3 expression in astrocytes may be an important reason for increased complement levels in the cerebrospinal fluid of HIV-infected patients. HIV-1 increased the C3 levels in astrocyte culture supernatants from 30 to up to 400 ng/ml. Signal transduction studies revealed that adenylate cyclase activation with upregulation of cyclic AMP is the central signalling pathway to mediate that increase. Furthermore, activity of protein kinase C was necessary for HIV induction of C3, since inhibition of protein kinase C by prolonged exposure to the phorbol ester tetradecanoyl phorbol acetate partly abolished the HIV effect.

Confocal microscopy analysis of human MDMs treated with myr- and myr+ Nef–FITC indicated that both proteins are internalized and localize in an intracytoplasmic punctate pattern and at the cell margin (Alessandrini et al., 2000; Olivetta et al., 2003) as described for cells that endogenously express the protein (Greenberg et al., 1997). The protein lacking the acceptor signal for myristoylation ($G^2 \rightarrow A$ Nef) is internalized, but loose at least in part the co-localization signal with the cell membrane (Alessandrini et al., 2000). The wt protein does not bind MDMs at 4 °C suggesting that it does not recognize specific receptor on the cell membrane, but it might be internalized exploiting the endocytic/pinocytotic MDMs machinery. Indeed Nef was shown to perturb model membranes opening an avenue for its translocation through cell membranes (Gerlach et al., 2010; Szilluweit et al., 2009).

All these experimental evidences suggest that extracellular Nef if present *in vivo* could induce specific cellular response in uninfected cells that might contribute directly and indirectly to the onset of AIDS. Indeed the presence of extracellular Nef in infected individuals has not been extensively evaluated. The viral protein has been found in the serum of some HIV-1 infected patients at concentration ranging from 1 to 10 ng/ml (Fujii et al., 1996c). This concentration might be even higher in the lymphonodal germinal centers, where virion-trapping dendritic cells, virion-infected $CD4^+$ T cells and macrophages are densely packed (Pantaleo et al., 1991). In addition antibodies directed against this viral protein have been found in HIV-1 infected seronegative individuals supporting the possible detection of extracellular molecule (Ameisen et al., 1989). Nef can be released in culture supernatant of 293, Jurkat and THP-1 cells transfected with $HIV\text{-}1_{NL4\text{-}3}$-, HIV-2- or SIV-Nef expressing vectors indicating that it can be secreted (James et al., 2004). The genetic characterization of Nef-induced secretion has identified that the N-terminal 70 amino acids were sufficient for induction of secretion of Nef- containing vescicles in several cell lines and identified critical aminoacid residues: (1) a basic cluster of four arginine residues (aa 17, 19, 21, 22), (2) the phosphofurin acidic cluster sequence (PACS; Glu62–65), and (3) a previously uncharacterized domain spanning amino acid residues 66–70 (VGFPV), which has been named the secretion modification region (SMR). Interestingly, *in vivo* analysis performed on infected follicles of lymphoid tissue showed the presence of Nef positive IgD^+ B cells at the

edge of the germinal center and in the interfollicular area even if these cells were unlikely to be infected by HIV-1 (Qiao et al., 2006). This observation suggested that Nef, produced inside the HIV infected cells via the transcription of the proviral genome, can be transferred *in vivo* to bystander non infected cells.

4. Nef transfer from infected to uninfected cells

The transfer of HIV viral proteins by nanotubes (Sowinski et al., 2008) and the transfer of Nef by vesicles (Campbell et al., 2008) has been recently documented. Interestingly in September 2009, Xu and collaborators (Xu et al., 2009) reported that HIV-1 infected macrophages form B cell–targeting conduits in response to Nef expression. These conduits translocate membrane-bound Nef and Nef-containing endosomes from macrophages to follicular B cells via a mechanism propelled by actin, mediated by Vav and dependent on GTPases. These results provided the evidence that infected cells can transfer the viral protein via cell to cell contact to uninfected cells. It appears that Nef-mediated activation of membrane trafficking is bidirectional, connecting endocytosis with exocytosis as occurs in activated T cells. Interestingly Nef expression induced an extensive secretory activity also in infected and, surprisingly, in non infected T cells, leading to the massive release of microvesicle clusters, a phenotype observed in vitro and in 36%–87% of primary $CD4^+$ T cells from HIV-infected individuals. Consistent with exocytosis in noninfected cells, Nef is transferred to bystander cells upon cell-to-cell contact and subsequently induces secretion in an Erk1/2-dependent manner. Thus, Nef alters membrane dynamics mimicking those of activated T cells and causing a transfer of infected cell signalling to bystander cells (Muratori et al., 2009). Based on these findings, it was hypothesized that Nef transfer could potentially explain the effects observed in bystander cells *in vivo*. In agreement with this hypothesis conduits-mediated shuttling of Nef from infected macrophages to B cells attenuated IgG2 and IgA class switching in systemic and intestinal lymphoid follicles suggesting that HIV-1 exploits intercellular 'highways' as a 'Trojan horse' to deliver Nef to B cells and evade specific humoral immunity systemically and at mucosal sites of entry. The stronger virus-specific IgG2 and IgA responses observed in Long Term Non Progressor infected by Nef-deficient HIV-1 further supports this possibility (Xu et al., 2009). In addition Lenassi et al. (Lenassi et al., 2010) demonstrated that the expression of Nef not only augmented the production of the exosomes from T cells (Jurkat, SupT1 and primary T cells) but also resulted in the packaging of Nef into these vesicles which, upon contact, leads to an activation-induced cell death in the resting CD4+ T lymphocytes (bystander cells). It is known that subsets of cellular proteins are specifically targeted to exosomes reflecting the site of their formation. Proteomic profiling of Nef exosomes originating from Jurkat and SupT1 cells indicates that Nef exosomes form at the plasma membrane in Jurkat and in Multivescicular Bodies in SupT cells. Instead Nef exosomes from PBLs represent a mixture of secreted vesicles of plasma membrane and late endosomal origin. Therefore, Nef increases the production of exosomes from several distinct cellular compartments. Nef release through exosomes was conserved also during HIV-1 infection of peripheral blood lymphocytes (PBLs). Thus, HIV-infected cells export Nef in bioactive vesicles that might facilitate the depletion of CD4+ T cells *in vivo*.
Figure 1 schematically summarizes Nef-induced effects and transfer in uninfected cells.

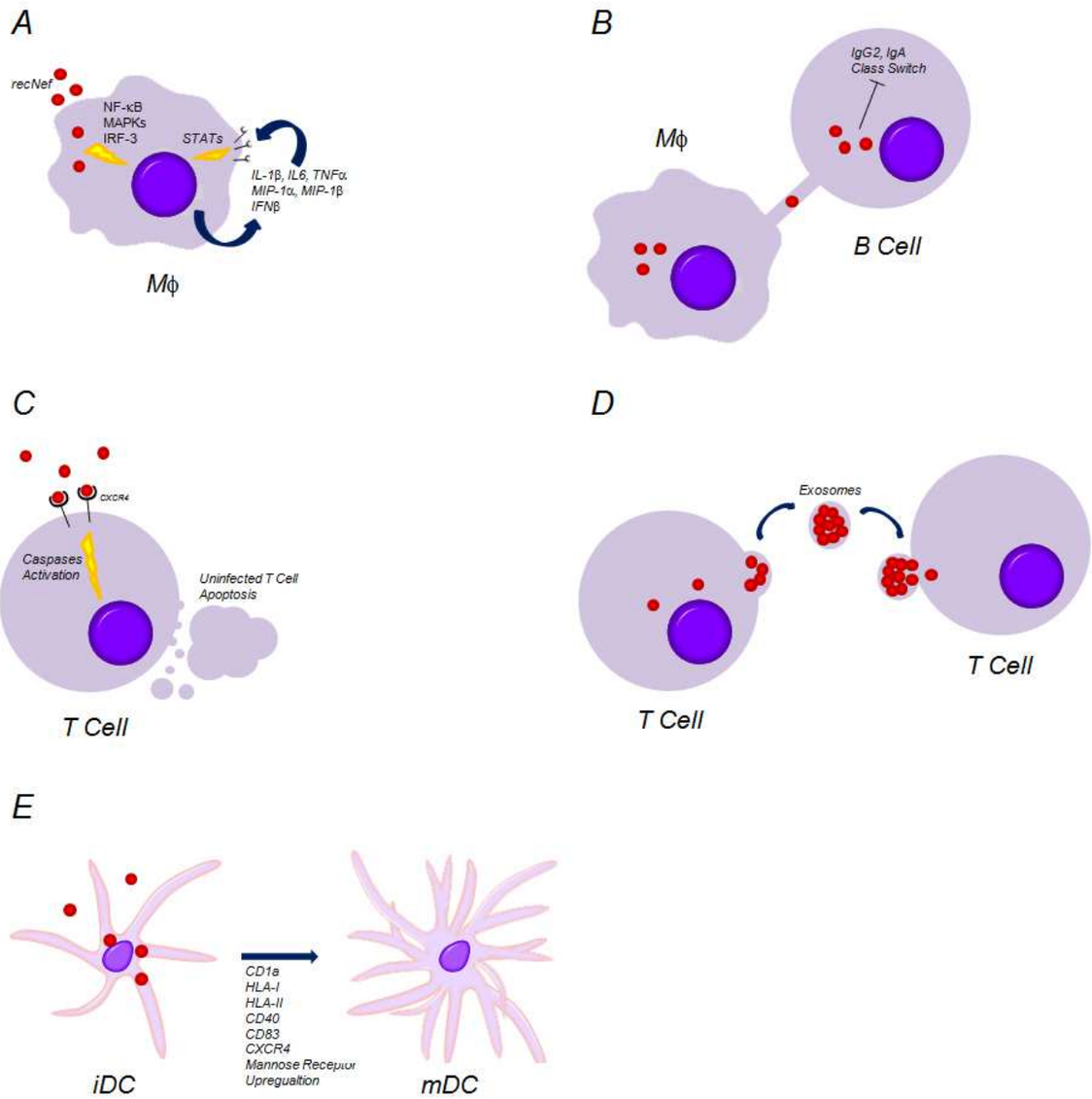


Fig. 1. Schematic representation of Nef-induced signalling and transfer in uninfected cells. A: Nef treatment of MDMs induces signal transduction events followed by production of inflammatory chemo/cytokines and IFNβ; B: infected MDMs transfer Nef via cellular protusions; C: exogenous Nef induces apoptosis in T cells via interaction with CXCR4; D: T cells expressing Nef release Nef-containing – exosomes that are transferred to bystander cells; E: extracellular Nef induces up regulation of specific maturation markers in immature DC.

5. Perspectives and future directions

In recent years, it has become apparent that many viruses induce signal transduction events in concert with virus entry and/or as a consequence of the intracellular expression and release of viral proteins. These events can trigger a variety of changes in the cell, including induction of apoptosis, cytoskeleton rearrangements and global reprogramming of cellular transcription to favour viral replication or persistence. At the same time the innate sensing

of some viral components by cells triggers signal transduction pathways to induce antiviral responses. At present, we are only beginning to decipher the mechanism and the consequences of these events to viral replication and pathogenesis in the host. As far as Nef concerns, its ability to induce exosome formation and cell to cell connection mediating its transfer and the transfer of signalling molecules between cells open new road to understand its complex role in HIV-1-host interaction and highlights the complexity of functions of biological molecules. How Nef is able to switch on signalling events in uninfected cells and the patho-physiological relevance of this phenomenon requires further investigations and might disclosure new therapeutic targets for treatment of seropositive patients.

6. References

Alessandrini, L., Santarcangelo, A. C., Olivetta, E., Ferrantelli, F., d'Aloja, P., Pugliese, K., Pelosi, E., Chelucci, C., Mattia, G., Peschle, C., Verani, P. & Federico, M. (2000). T-tropic human immunodeficiency virus (HIV) type 1 Nef protein enters human monocyte-macrophages and induces resistance to HIV replication: a possible mechanism of HIV T-tropic emergence in AIDS. *Journal of General Virology,* Vol.81, No.12, (December 2000), pp. 2905-2917, ISSN 0022-1317

Ameisen, J., Guy, B., Chamaret, S., Loche, M., Mouton, Y., Neyrinck, J., Khalife, J., Leprevost, C., Beaucaire, G. & Boutillon, C. (1989). Antibodies to the nef protein and to nef peptides in HIV-1-infected seronegative individuals. *AIDS Research and Human Retroviruses*, Vol.5, No.3, (June 1989), pp. 279-291, ISSN 0889-2229

Anderson, S., Shugars, D. C., Swanstrom, R. & Garcia, J. V. (1993). Nef from primary isolates of human immunodeficiency virus type 1 suppresses surface CD4 expression in human and mouse T cells. *Journal of Virology*, Vol.67, No.8, (August 1993), pp. 4923-4931, ISSN 0022-538X

Ankel, H., Capobianchi, M. R., Castilletti, C. & Dianzani, F. (1994). Interferon induction by HIV glycoprotein 120: role of the V3 loop. *Virology*, Vol.205, No.1, (November 1994), pp. 34-43, ISSN 0042-6822

Arold, S., Franken, P., Strub, M. P., Hoh, F., Benichou, S., Benarous, R. & Dumas, C. (1997). The crystal structure of HIV-1 Nef protein bound to the Fyn kinase SH3 domain suggests a role for this complex in altered T cell receptor signaling. *Structure*, Vol.5, No.10, (October 1997), pp. 1361-1372 ISSN 0910-8270

Arold, S. T. & Baur, A. S. (2001). Dynamic Nef and Nef dynamics: how structure could explain the complex activities of this small HIV protein. *Trends in Biochemical Sciences*, Vol.26, No.6, (June2001), pp. 356-363, ISSN 0968-0004

Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science*, Vol.220, No.4599, (May 1983), pp. 868-871, ISSN 0193-4511

Bentham, M., Mazaleyrat, S. & Harris, M. (2006). Role of myristoylation and N-terminal basic residues in membrane association of the human immunodeficiency virus type 1 Nef protein. *Journal of General Virology*, Vol.87, No.3, (May 2006), pp. 563-571, ISSN 0022-1317

Blagoveshchenskaya, A. D., Thomas, L., Feliciangeli, S. F., Hung, C. H. & Thomas, G. (2002). HIV-1 Nef downregulates MHC-I by a PACS-1- and PI3K-regulated ARF6

endocytic pathway. *Cell*, Vol.111, No.6, (December 2002), pp. 853-866, ISSN 0092-8674

Breuer, S., Gerlach, H., Kolaric, B., Urbanke, C., Opitz, N. & Geyer, M. (2006). Biochemical Indication for Myristoylation-Dependent Conformational Changes in HIV-1 Nef. *Biochemistry*, Vol.45, No.7, (Febraury 2006), pp. 2339-2349, ISSN 0006-2960

Brigino, E., Haraguchi, S., Koutsonikolis, A., Cianciolo, G. J., Owens, U., Good, R. A. & Day, N. K. (1997). Interleukin 10 is induced by recombinant HIV-1 Nef protein involving the calcium/calmodulin-dependent phosphodiesterase signal transduction pathway. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.94, No.7, (April 1997), pp. 3178-3182, ISSN 0027-8424

Burtey, A., Rappoport, J. Z., Bouchet, J., Basmaciogullari, S., Guatelli, J., Simon, S. M., Benichou, S. & Benmerah, A. (2007). Dynamic interaction of HIV-1 Nef with the clathrin-mediated endocytic pathway at the plasma membrane. *Traffic*, Vol.8, No.1, (January 2007), pp. 61-76, ISSN 1398-9219

Campbell, T. D., Khan, M., Huang, M. B., Bond, V. C. & Powell, M. D. (2008). HIV-1 Nef protein is secreted into vesicles that can fuse with target cells and virions. *Ethnicity and disease*, Vol.18, (Spring), pp. S2-14-19, ISSN 1049-510X

Capobianchi, M. R., Ameglio, F., Cordiali Fei, P., Castilletti, C., Mercuri, F., Fais, S. & Dianzani, F. (1993). Coordinate induction of interferon alpha and gamma by recombinant HIV-1 glycoprotein 120. *AIDS Research and Human Retroviruses*, Vol.9, No.10, (October 1993), pp. 957-962, ISSN 0889-2229

Capobianchi, M. R., Ankel, H., Ameglio, F., Paganelli, R., Pizzoli, P. M. & Dianzani, F. (1992). Recombinant glycoprotein 120 of human immunodeficiency virus is a potent interferon inducer. *AIDS Research and Human Retroviruses*, Vol.8, No.5, (May 1992), pp. 575-579, ISSN 0889-2229

Chaudhry, A., Das, S. R., Hussain, A., Mayor, S., George, A., Bal, V., Jameel, S. & Rath, S. (2005). The Nef protein of HIV-1 induces loss of cell surface costimulatory molecules CD80 and CD86 in APCs. *Journal of Immunology*, Vol.175, No.7, (October 2005), pp. 4566-4574, ISSN 0022-1767

Creery, D., Angel, J. B., Aucoin, S., Weiss, W., Cameron, W. D., Diaz-Mitoma, F. & Kumar, A. (2002). Nef protein of human immunodeficiency virus and lipopolysaccharide induce expression of CD14 on human monocytes through differential utilization of interleukin-10. *Clinical and diagnostic laboratory immunology*, Vol.9, No.6, (November 2002), pp. 1212-1221, ISSN 1071-412X

Daniel, M. D., Kirchhoff, F., Czajak, S. C., Sehgal, P. K. & Desrosiers, R. C. (1992). Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. *Science*, Vol.258, No.5090, (December 1992), pp. 1938-1941, ISSN: 0036-8075

Das, S. R. & Jameel, S. (2005). Biology of the HIV Nef protein. *Indian Journal of Medical Research*, Vol.121, No.4, (April 2005), pp. 315-332, ISSN:0971-5916

Deacon, N. J., Tsykin, A., Solomon, A., Smith, K., Ludford-Menting, M., Hooker, D. J., McPhee, D. A., Greenway, A. L., Ellett, A., Chatfield, C. & al, e. (1995). Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. *Science*, Vol.270, No.5238, (November 1995), pp. 988-991, ISSN: 0036-8075

Dennis, C. A., Baron, A., Grossmann, J. G., Mazaleyrat, S., Harris, M. & Jaeger, J. (2005). Co-translational myristoylation alters the quaternary structure of HIV-1 Nef in solution. *Proteins*, Vol.60, No.4, (September 2005), pp. 658-669, ISSN:0887-3585

Doehle, B. P., Hladik, F., McNevin, J. P., McElrath, M. J. & Gale, M. (2009). Human immunodeficiency virus type 1 mediates global disruption of innate antiviral signaling and immune defenses within infected cells. *Journal of Virology*, Vol.83, No.83, (October 2009), pp. 10395-10405, ISSN 0022-538X

Doms, R. W. & Trono, D. (2000). The plasma membrane as a combat zone in the HIV battlefield. *Genes and Development*, Vol.14, No.21, (November 2000), pp. 2677-2688, ISSN 0890-9369

Federico, M., Percario, Z., Olivetta, E., Fiorucci, G., Muratori, C., Micheli, A., Romeo, G. & Affabris, E. (2001). HIV-1 Nef activates STAT1 in human monocytes/macrophages through the release of soluble factors. *Blood*, Vol.98, No.9, (November 2001), pp. 2752-2761, ISSN 0006-4971

Foster, J.L, Denial, S.J., Temple, B.R.S. & Garcia, J.V. (2011) Mechanisms of HIV-1 Nef Function and Intracellular Signaling. J Neuroimmune Pharmacol (June 2011) Vol.6, No.2, pp. 230–246 ISSN 1557-1890

Fujii, Y., Otake, K., Tashiro, M. & Adachi, A. (1996a). Human immunodeficiency virus type 1 Nef protein on the cell surface is cytocidal for human CD4+ T cells. *FEBS Letters*, Vol.393, No.1, (September 1996), pp. 105-108, ISSN 0014-5793

Fujii, Y., Otake, K., Tashiro, M. & Adachi, A. (1996b). In vitro cytocidal effects of human immunodeficiency virus type 1 Nef on unprimed human CD4+ T cells without MHC restriction. *Journal of General Virology*, Vol.77, No.12, pp. 2943-2951, ISSN 0022-1317

Fujii, Y., Otake, K., Tashiro, M. & Adachi, A. (1996c). Soluble Nef antigen of HIV-1 is cytotoxic for human CD4+ T cells. *FEBS Letters*, Vol.393, No.1, (September 1996), pp. 93-96, ISSN 0014-5793

Fujinaga, K., Zhong, Q., Nakaya, T., Kameoka, M., Meguro, T., Yamada, K. & Ikuta, K. (1995). Extracellular Nef protein regulates productive HIV-1 infection from latency. *Journal of Immunology*, Vol.155, No.11, (December 1995), pp.5289-5298, ISSN 0022-1767

Geleziunas, R., Miller, M. D. & Greene, W. C. (1996). Unraveling the function of HIV type 1 Nef. *AIDS Research and Human Retroviruses*, Vol.12, No.17, (November 1996), pp. 1579-1582, ISSN 0889-2229

Geleziunas, R., Xu, W., Takeda, K., Ichijo, H. & Greene, W. C. (2001). HIV-1 Nef inhibits ASK1-dependent death signalling providing a potential mechanism for protecting the infected host cell. *Nature*, Vol.410, No.6830, (April 2001), pp. 834-838, ISSN 0028-0836

Gerlach, H., Laumann, V., Martens, S., Becker, C., Goody, R. & Geyer, M. (2010). HIV-1 Nef membrane association depends on charge, curvature, composition and sequence. *Nature chemical biology*, Vol.6, No.1, (January 2010), pp. 46-53 ISSN 1552-4469

Gessani, S., Puddu, P., Varano, B., Borghi, P., Conti, L., Fantuzzi, L. & Belardelli, F. (1994). Induction of beta interferon by human immunodeficiency virus type 1 and its gp120 protein in human monocytes-macrophages: role of beta interferon in restriction of virus replication. *Journal of Virology*, Vol.68, No.3, (March 1994), pp.1983-1986 ISSN 0022-538X

Geyer, M., Fackler, O. T. & Peterlin, B. M. (2001). Structure-function relationships in HIV-1 Nef. *EMBO Reports*, Vol.2, No.7, (July 2001), pp. 580-585, ISSN 1469-221X

Geyer, M., Munte, C. E., Schorr, J., Kellner, R. & Kalbitzer, H. R. (1999). Structure of the anchor-domain of myristoylated and non-myristoylated HIV-1 Nef protein. *Journal of Molecular Biology*, Vol.289, No.1, (May 1999), pp.123-138, ISSN 0022-2836

Greenberg, M. E., Bronson, S., Lock, M., Neumann, M., Pavlakis, G. N. & Skowronski, J. (1997). Co-localization of HIV-1 Nef with the AP-2 adaptor protein complex correlates with Nef-induced CD4 down-regulation. *EMBO Journal*, Vol.16, No.23, (December 1997), pp. 6964-6976, ISSN 0261-4189

Greenway, A. L., McPhee, D. A., Allen, K., Johnstone, R., Holloway, G., Mills, J., Azad, A., Sankovich, S. & Lambert, P. (2002). Human immunodeficiency virus type 1 Nef binds to tumor suppressor p53 and protects cells against p53-mediated apoptosis. *Journal of Virology*, Vol.76, No.6, (March 2002), pp. 2692-2702, ISSN 0022-538X

Grzesiek, S., Bax, A., Hu, J. S., Kaufman, J., Palmer, I., Stahl, S. J., Tjandra, N. & Wingfield, P. T. (1997). Refined solution structure and backbone dynamics of HIV-1 Nef. *Protein Science*, Vol.6, No.6, (June 1997), pp.1248-1263, ISSN 0961-8368

Gulizia, R. J., Collman, R. G., Levy, J. A., Trono, D. & Mosier, D. E. (1997). Deletion of nef slows but does not prevent CD4-positive T-cell depletion in human immunodeficiency virus type 1-infected human-PBL-SCID mice. *Journal of Virology*, Vol.71, No.5, (May 1997), pp. 4161-4164, ISSN 0022-538X

Hanna, Z., Kay, D. G., Rebai, N., Guimond, A., Jothy, S. & Jolicoeur, P. (1998). Nef harbors a major determinant of pathogenicity for an AIDS-like disease induced by HIV-1 in transgenic mice. *Cell*, Vol.95, No.2, (October 1998), pp. 163-175, ISSN 0092-8674

Huang, M. B., Jin, L. L., James, C. O., Khan, M., Powell, M. D. & Bond, V. C. (2004). Characterization of Nef-CXCR4 interactions important for apoptosis induction. *Journal of Virology*, Vol.78, No.20, (October 2004), pp. 11084-11096, ISSN 0022-538X

James, C. O., Huang, M. B., Khan, M., Garcia-Barrio, M., Powell, M. D. & Bond, V. C. (2004). Extracellular Nef protein targets CD4+ T cells for apoptosis by interacting with CXCR4 surface receptors. *Journal of Virology*, Vol.78, No.6, (March 2004), pp. 3099-3109, ISSN 0022-538X

Kaminchik, J., Margalit, R., Yaish, S., Drummer, H., Amit, B., Sarver, N., Gorecki, M. & Panet, A. (1994). Cellular distribution of HIV type 1 Nef protein: identification of domains in Nef required for association with membrane and detergent-insoluble cellular matrix. *AIDS Research and Human Retroviruses*, Vol.10, No.8, (August 1994), pp.1003-1010, ISSN 0889-2229

Kestler, H. W. r., Ringler, D. J., Mori, K., Panicali, D. L., Sehgal, P. K., Daniel, M. D. & Desrosiers, R. C. (1991). Importance of the nef gene for maintenance of high virus loads and for development of AIDS. *Cell*, Vol.65, No.4, (May 1991), pp. 651-662, ISSN 0092-8674

Kirchhoff, F., Greenough, T. C., Brettler, D. B., Sullivan, J. L. & Desrosiers, R. C. (1995). Brief report: absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. *The New England Journal of Medicine*, Vol.332, No.4, (January 1995), pp. 228-232, ISSN 0028-4793

Lama, J. & Ware, C. F. (2000). Human immunodeficiency virus type 1 Nef mediates sustained membrane expression of tumor necrosis factor and the related cytokine LIGHT on activated T cells. *Journal of Virology*, Vol.74, No.20, (October 2000), pp. 9396-9402, ISSN 0022-538X

Lee, C. H., Saksela, K., Mirza, U. A., Chait, B. T. & Kuriyan, J. (1996). Crystal structure of the conserved core of HIV-1 Nef complexed with a Src family SH3 domain. *Cell*, Vol.85, No.6, (June 1996), pp. 931-942, ISSN 0092-8674

Lehmann, M. H., Walter, S., Ylisastigui, L., Striebel, F., Ovod, V., Geyer, M., Gluckman, J. C. & Erfle, V. (2006). Extracellular HIV-1 Nef increases migration of monocytes.

Experimental Cell Research, Vol.312, No.18, (November 2006), pp. 3659-3668, ISSN 0014-4827

Lenassi, M., Cagney, G., Liao, M., Vaupotic, T., Bartholomeeusen, K., Cheng, Y., Krogan, N. J., Plemenitas, A. & Peterlin, B. M. (2010). HIV Nef is secreted in exosomes and triggers apoptosis in bystander CD4+ T cells. *Traffic*, Vol.11, No.1, (January 2010), pp. 110-122, ISSN 1600-0854

Madrid, R., Janvier, K., Hitchin, D., Day, J., Coleman, S., Noviello, C., Bouchet, J., Benmerah, A., Guatelli, J. & Benichou, S. (2005). Nef-induced alteration of the early/recycling endosomal compartment correlates with enhancement of HIV-1 infectivity. *Journal of Biological Chemistry*, Vol.280, No.6, (Febraury 2005), pp. 5032-5044, ISSN 0021-9258

Malim, M. H. & Emerman, M. (2008). HIV-1 accessory proteins-ensuring viral survival in a hostile environment. *Cell Host and Microbe*, Vol.3, No.6, (June 2008), pp. 388-398, ISSN 1934-6069

Mangino, G., Percario, Z., Fiorucci, G., Vaccari, G., Manrique, S., Romeo, G., Federico, M., Geyer, M. & Affabris, E. (2007). In vitro treatment of human monocytes/macrophages with myristoylated recombinant Nef of human immunodeficiency virus type 1 leads to the activation of mitogen-activated protein kinases, IkappaB kinases, and interferon regulatory factor 3 and to the release of beta interferon. *Journal of Virology*, Vol.81, No.6, (March 2007), pp. 2777-2791, ISSN 0022-538X

Muratori, C., Cavallin, L. E., Krätzel, K., Tinari, A., De Milito, A., Fais, S., D'Aloja, P., Federico, M., Vullo, V., Fomina, A., Mesri, E. A., Superti, F. & Baur, A. S. (2009). Massive secretion by T cells is caused by HIV Nef in infected cells and by Nef transfer to bystander cells. *Cell Host and Microbe*, Vol.6, No.3, (September 2009), pp. 218-230, ISSN 1931-3128

Okada, H., Morikawa, S. & Tashiro, M. (1998). HIV-1 Nef binding protein expressed on the surface of murine blood cells. *Medical microbiology and immunology (Berlin)* 186, 201-207.

Okada, H., Takei, R. & Tashiro, M. (1997). HIV-1 Nef protein-induced apoptotic cytolysis of a broad spectrum of uninfected human blood cells independently of CD95(Fas). *FEBS Letters*, Vol.414, No.4, (March 1997), pp. 603-606, ISSN 0300-8584

Olivetta, E., Percario, Z., Fiorucci, G., Mattia, G., Schiavoni, I., Dennis, C., Jager, J., Harris, M., Romeo, G., Affabris, E. & Federico, M. (2003). HIV-1 Nef induces the release of inflammatory factors from human monocyte/macrophages: involvement of Nef endocytotic signals and NF-kappaB activation. *Journal of Immunology*, Vol.170, No.4, (February 2003), pp. 1716-1727, ISSN 0022-1767

Pantaleo, G., Graziosi, C., Butini, L., Pizzo, P. A., Schnittman, S. M., Kotler, D. P. & Fauci, A. S. (1991). Lymphoid organs function as major reservoirs for human immunodeficiency virus. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.88, No.21, (November 1991), pp. 9838-9842, ISSN 0027-8424

Peng, G., Lei, K. J., Jin, W., Greenwell-Wild, T. & Wahl, S. M. (2006). Induction of APOBEC3 family proteins, a defensive maneuver underlying interferon-induced anti-HIV-1 activity. *Journal of Experimental Medicine*, Vol.203, No.1, (January 2006), pp. 41-46, ISSN 0022-1007

Percario, Z., Olivetta, E., Fiorucci, G., Mangino, G., Peretti, S., Romeo, G., Affabris, E. & Federico, M. (2003). Human immunodeficiency virus type 1 (HIV-1) Nef activates STAT3 in primary human monocyte/macrophages through the release of soluble factors: involvement of Nef domains interacting with the cell endocytotic

machinery. *Journal of Leukocyte Biology*, Vol.74, No.5, (November 2003), pp. 821-832, ISSN 0741-5400

Peterlin, B. (2006). Nef: out and in? *Nature Immunology*, Vol.7, No.3, (March 2006), pp. 229-230, ISSN 1529-2908

Peterlin, B. M. & Trono, D. (2003). Hide, shield and strike back: how HIV-infected cells avoid immune eradication. *Nature reviews. Immunology*, Vol.3, No.2, (Febraury 2003), pp. 97-107, ISSN 1474-1733

Pitha-Rowe, I. F. & Pitha, P. M. (2007). Viral defense, carcinogenesis and ISG15: novel roles for an old ISG. *Cytokine Growth Factor Rev*, Vol.18, No.5-6, (October-December 2007), pp. 409-417, ISSN 1359-6101

Qiao, X., He, B., Chiu, A., Knowles, D. M., Chadburn, A. & Cerutti, A. (2006). Human immunodeficiency virus 1 Nef suppresses CD40-dependent immunoglobulin class switching in bystander B cells. *Nature Immunology*, Vol.7, No.3, (March 2006), pp. 302-310, ISSN 1529-2908

Quaranta, M. G., Camponeschi, B., Straface, E., Malorni, W. & Viora, M. (1999). Induction of Interleukin-15 production by HIV-1 Nef protein: a role in the proliferation of uninfected cells. *Experimental Cell Research*, Vol.250, No.1, (July 1999), pp. 112-121, ISSN 0014-4827

Quaranta, M. G., Mattioli, B., Giordani, L. & Viora, M. (2009). Immunoregulatory effects of HIV-1 Nef protein. *Biofactors*, Vol.35, No.2, (March-April 2009), pp. 169-174, ISSN 0951-6433

Quaranta, M. G., Mattioli, B., Spadaro, F., Straface, E., Giordani, L., Ramoni, C., Malorni, W. & Viora, M. (2003). HIV-1 Nef triggers Vav-mediated signaling pathway leading to functional and morphological differentiation of dendritic cells. The *FASEB Journal*, Vol.17, No.14, (November 2003), pp. 2025-2036, ISSN 0892-6638

Quaranta, M. G., Tritarelli, E., Giordani, L. & Viora, M. (2002). HIV-1 Nef induces dendritic cell differentiation: a possible mechanism of uninfected CD4(+) T cell activation. *Experimental Cell Research*, Vol.275, No.2, (May 2002), pp. 243-254, ISSN 0014-4827

Renkema, G. H. & Saksela, K. (2000). Interactions of HIV-1 NEF with cellular signal transducing proteins. *Frontiers in bioscience*, Vol.5, (Febraury 2000), pp. D268-283, ISSN 1093-9946

Roeth, J. F. & Collins, K. L. (2006). Human immunodeficiency virus type 1 Nef: adapting to intracellular trafficking pathways. *Microbiology and molecular biology reviews*, Vol.70, No.2, (June 2006), pp. 548-563, ISSN 1092-2172

Schindler, M., Münch, J., Kutsch, O., Li, H., Santiago, M. L., Bibollet-Ruche, F., Müller-Trutwin, M. C., Novembre, F. J., Peeters, M., Courgnaud, V., Bailes, E., Roques, P., Sodora, D. L., Silvestri, G., Sharp, P. M., Hahn, B. H. & Kirchhoff, F. (2006). Nef-mediated suppression of T cell activation was lost in a lentiviral lineage that gave rise to HIV-1. *Cell*, Vol.125, No.6, (June 2006), pp. 1055-1067, ISSN 0092-8674

Schindler, M., Würfl, S., Benaroch, P., Greenough, T. C., Daniels, R., Easterbrook, P., Brenner, M., Münch, J. & Kirchhoff, F. (2003). Down-modulation of mature major histocompatibility complex class II and up-regulation of invariant chain cell surface expression are well-conserved functions of human and simian immunodeficiency virus nef alleles. *Journal of Virology*, Vol.77, No.19, (October 2003), pp. 10548-10556, ISSN 0022-538X

Schwartz, O., Marechal, V., Le Gall, S., Lemonnier, F. & Heard, J. M. (1996). Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein. *Nature Medicine*, Vol.2, No.3, (March 1996), pp. 338-342, ISSN 1078-8956

Simmons, A., Aluvihare, V. & McMichael, A. (2001). Nef triggers a transcriptional program in T cells imitating single-signal T cell activation and inducing HIV virulence mediators. *Immunity*, Vol.14, No.6, (June 2001), pp. 763-777, ISSN 1074-7613

Sol-Foulon, N., Moris, A., Nobile, C., Boccaccio, C., Engering, A., Abastado, J. P., Heard, J. M., van Kooyk, Y. & Schwartz, O. (2002). HIV-1 Nef-induced upregulation of DC-SIGN in dendritic cells promotes lymphocyte clustering and viral spread. *Immunity*, Vol.16, No.1, (Janauary 2002), pp. 145-155, ISSN 1074-7613

Sowinski, S., Jolly, C., Berninghausen, O., Purbhoo, M. A., Chauveau, A., Köhler, K., Oddos, S., Eissmann, P., Brodsky, F. M., Hopkins, C., Onfelt, B., Sattentau, Q. & Davis, D. M. (2008). Membrane nanotubes physically connect T cells over long distances presenting a novel route for HIV-1 transmission. *Nature Cell Biology*, Vol.10, No.2, (Febraury 2008), pp. 211-219, ISSN 1476-4679

Speth, C., Schabetsberger, T., Mohsenipour, I., Stöckl, G., Würzner, R., Stoiber, H., Lass-Flörl, C. & Dierich, M. P. (2002). Mechanism of human immunodeficiency virus-induced complement expression in astrocytes and neurons. *Journal of Virology*, Vol.76, No.7, (April 2002), pp. 3179-3188, ISSN 0022-538X

Stacey, A. R., Norris, P. J., Qin, L., Haygreen, E. A., Taylor, E., Heitman, J., Lebedeva, M., DeCamp, A., Li, D., Grove, D., Self, S. G. & Borrow, P. (2009). Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. *Journal of Virology*, Vol.83, No.8, (April 2009), pp. 3719-3733, ISSN 0022-538X

Stove, V., Van de Walle, I., Naessens, E., Coene, E., Stove, C., Plum, J. & Verhasselt, B. (2005). Human immunodeficiency virus Nef induces rapid internalization of the T-cell coreceptor CD8alphabeta. *Journal of Virology*, Vol.79, No.17, (September 2005), pp. 11422-11433, ISSN 0022-538X

Stumptner-Cuvelette, P., Morchoisne, S., Dugast, M., Le Gall, S., Raposo, G., Schwartz, O. & Benaroch, P. (2001). HIV-1 Nef impairs MHC class II antigen presentation and surface expression. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.98, No.21, (October 2001), pp. 12144-12149, ISSN 0027-8424

Swingler, S., Brichacek, B., Jacque, J. M., Ulich, C., Zhou, J. & Stevenson, M. (2003). HIV-1 Nef intersects the macrophage CD40L signalling pathway to promote resting-cell infection. *Nature*, Vol.424, No.6945, (July 2003), pp. 213-219, ISSN 0028-0836

Swingler, S., Mann, A., Jacque, J., Brichacek, B., Sasseville, V. G., Williams, K., Lackner, A. A., Janoff, E. N., Wang, R., Fisher, D. & Stevenson, M. (1999). HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. *Nature Medicine*, Vol.5, No.9, (September 1999), pp. 997-1003, ISSN 1078-8956

Szebeni, J., Dieffenbach, C., Wahl, S. M., Venkateshan, C. N., Yeh, A., Popovic, M., Gartner, S., Wahl, L. M., Peterfy, M., Friedman, R. M. & al, e. (1991). Induction of alpha interferon by human immunodeficiency virus type 1 in human monocyte-macrophage cultures. *Journal of Virology*, Vol.65, No.11, (November 1991), pp. 6362-6364, ISSN 0022-538X

Szilluweit, R., Boll, A., Lukowski, S., Gerlach, H., Fackler, O., Geyer, M. & Steinem, C. (2009). HIV-1 Nef perturbs artificial membranes: investigation of the contribution of the

myristoyl anchor. *Biophysical journal*, Vol.96, No.8, (April 2009), pp. 3242-3250, ISSN 1542-0086

Tobiume, M., Fujinaga, K., Suzuki, S., Komoto, S., Mukai, S. & Ikuta, K. (2002). Extracellular Nef protein activates signal transduction pathway from Ras to mitogen-activated protein kinase cascades that leads to activation of human immunodeficiency virus from latency. *AIDS Research and Human Retroviruses*, Vol.18, No.6, (April 2002), pp. 461-467, ISSN 0889-2229

Varin, A., Manna, S. K., Quivy, V., Decrion, A. Z., Van Lint, C., Herbein, G. & Aggarwal, B. B. (2003). Exogenous Nef protein activates NF-kappa B, AP-1, and c-Jun N-terminal kinase and stimulates HIV transcription in promonocytic cells. Role in AIDS pathogenesis. *Journal of Biological Chemistry*, Vol.278, No.4, (Janauary 2003), pp. 2219-2227, ISSN 0021-9258

Witte, V., Laffert, B., Rosorius, O., Lischka, P., Blume, K., Galler, G., Stilper, A., Willbold, D., D'Aloja, P., Sixt, M., Kolanus, J., Ott, M., Kolanus, W., Schuler, G. & Baur, A. S. (2004). HIV-1 Nef mimics an integrin receptor signal that recruits the polycomb group protein Eed to the plasma membrane. *Molecular Cell*, Vol.13, No.2, (Janaury 2004), pp. 179-190, ISSN 1097-2765

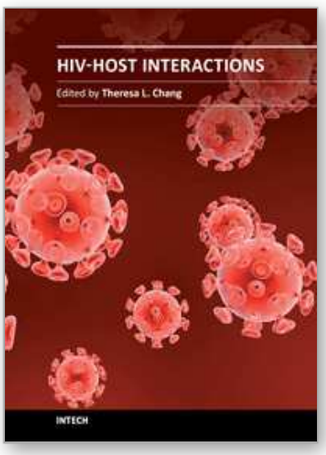
Wolf, D., Witte, V., Laffert, B., Blume, K., Stromer, E., Trapp, S., d'Aloja, P., Schurmann, A. & Baur, A. S. (2001). HIV-1 Nef associated PAK and PI3-kinases stimulate Akt-independent Bad-phosphorylation to induce anti-apoptotic signals. *Nature Medicine*, Vol.7, No.11, (November 2001), pp. 1217-1224, ISSN 1078-8956

Wu, Y. & Marsh, J. W. (2001). Selective transcription and modulation of resting T cell activity by preintegrated HIV DNA. *Science*, Vol.293, No.5534, (August 2001), pp. 1503-1506, ISSN 0036-8075

Xu, W., Santini, P. A., Sullivan, J. S., He, B., Shan, M., Ball, S. C., Dyer, W. B., Ketas, T. J., Chadburn, A., Cohen-Gould, L., Knowles, D. M., Chiu, A., Sanders, R. W., Chen, K. & Cerutti, A. (2009). HIV-1 evades virus-specific IgG2 and IgA responses by targeting systemic and intestinal B cells via long-range intercellular conduits. *Nature Immunol,* Vol.10, No.9, (September 2009), pp. 1008-1017, ISSN 1529-2908

Xu, X. N., Laffert, B., Screaton, G. R., Kraft, M., Wolf, D., Kolanus, W., Mongkolsapay, J., McMichael, A. J. & Baur, A. S. (1999). Induction of Fas ligand expression by HIV involves the interaction of Nef with the T cell receptor zeta chain. *Journal of Experimental Medicine,* Vol.189, No.9, (May 1999), pp. 1489-1496, ISSN 0022-1007

Xu, X. N., Screaton, G. R., Gotch, F. M., Dong, T., Tan, R., Almond, N., Walker, B., Stebbings, R., Kent, K., Nagata, S., Stott, J. E. & McMichael, A. J. (1997). Evasion of cytotoxic T lymphocyte (CTL) responses by nef-dependent induction of Fas ligand (CD95L) expression on simian immunodeficiency virus-infected cells. *Journal of Experimental Medicine,* Vol.186, No.1, (July 1997), pp. 7-16, ISSN 0022-1007



HIV-Host Interactions

Edited by Dr. Theresa Li-Yun Chang

ISBN 978-953-307-442-9
Hard cover, 364 pages
Publisher InTech
Published online 02, November, 2011
Published in print edition November, 2011

HIV remains the major global health threat, and neither vaccine nor cure is available. Increasing our knowledge on HIV infection will help overcome the challenge of HIV/AIDS. This book covers several aspects of HIV-host interactions in vitro and in vivo. The first section covers the interaction between cellular components and HIV proteins, Integrase, Tat, and Nef. It also discusses the clinical relevance of HIV superinfection. The next two chapters focus on the role of innate immunity including dendritic cells and defensins in HIV infection followed by the section on the impact of host factors on HIV pathogenesis. The section of co-infection includes the impact of Human herpesvirus 6 and Trichomonas vaginalis on HIV infection. The final section focuses on generation of HIV molecular clones that can be used in macaques and the potential use of cotton rats for HIV studies.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Zulema A. Percario, Giorgio Mangino, Valentina Gallo, Maria Vincenza Chiantore, Gianna Fiorucci, Giovanna Romeo and Elisabetta Affabris (2011). HIV-1 Nef Transfer and Intracellular Signalling in Uninfected Cells, HIV-Host Interactions, Dr. Theresa Li-Yun Chang (Ed.), ISBN: 978-953-307-442-9, InTech, Available from: http://www.intechopen.com/books/hiv-host-interactions/hiv-1-nef-transfer-and-intracellular-signalling-in-uninfected-cells

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