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Expression of simian immunodeficiency virus Nef protein in CD4$^+$ T cells leads to a molecular profile of viral persistence and immune evasion

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Abstract

The Nef protein of human immunodeficiency virus and simian immunodeficiency virus is expressed early in infection and plays an important role in disease progression in vivo. In addition, Nef has been shown to modulate cellular functions. To decipher Nef-mediated changes in gene expression, we utilized DNA microarray analysis to elucidate changes in gene expression in a Jurkat CD4$^+$ T-cell line stably expressing SIV-Nef protein under the control of an inducible promoter. Our results showed that genes associated with antigen presentation including members of the T-cell receptor and major histocompatibility class 1 complex were consistently down-regulated at the transcript level in SIV-Nef-expressing cells. In addition, Nef induced a transcriptional profile of cell-cycle-related genes that support the survival of Nef-expressing cells. Furthermore, Nef enhanced the transcription of genes encoding enzymes and factors that catalyze the biosynthesis of membrane glycolipids and phospholipids. In conclusion, gene expression profiling showed that SIV-Nef induces a transcriptional profile in CD4$^+$ T cells that promotes immune evasion and cell survival, thus facilitating viral persistence.
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Keywords: SIV-Nef; Immune evasion; DNA microarray; Antigen presentation; Viral persistence

Introduction

Retroviruses, including human and simian immunodeficiency viruses (HIV and SIV), have evolved elaborate strategies to evade host immune surveillance in order to replicate and establish a persistent infection. One of the strategies employed by HIV and SIV is the impairment of antigen presentation by down-regulation of host MHC molecules. In addition, HIV infection subverts the apoptotic machinery in both infected and uninfected cells, thereby facilitating the survival of virally infected cells. Among the viral accessory proteins, Nef has been shown to contribute to immune evasion. The *nef* gene of HIV and SIV encodes 25- to 27-kDa myristoylated protein that is expressed early during the virus life cycle and plays a crucial role in disease progression (Deacon et al., 1995; Kestler et al., 1991; Kirchhoff et al., 1995). Multiple cellular functions have been attributed to Nef protein. Down-regulation of CD4 molecules from the cell surface is the most clearly documented function of Nef involving direct bridging of the cytoplasmic tail of CD4 to an adaptor protein which targets it for endocytosis and degradation (Aiken et al., 1994; Bandres et al., 1995; Guy et al., 1987; Lama et al., 1999; Mariani and Skowronski, 1993; Rhee and Marsh, 1994). The down-regulation of CD4 molecules is thought to facilitate virion release (Ross et al., 1999). Nef is also known to modulate T-cell activation through the interaction between Nef and signal transduction proteins (Baur et al., 1994; Sawai et al., 1994; Skowronski et al., 1993).

The most pathophysiologically significant effect of Nef on CD4$^+$ T cells expressing Nef and virally infected cells is the evasion of immune responses. Nef down-regulates major histocompatibility complex class I (MHC-I) molecules from the cell surface (Greenberg et al., 1998; Schwartz et al., 1996; Swigut et al., 2000). This function of Nef involves indirect interaction with the cytoplasmic tail of MHC-I A and B molecules leading to endocytosis and confers partial protection from recognition and lysis by cytotoxic T cells (Collins et al., 1998; Greenberg et al., 1998; Schwartz et al., 1996). Nef has also been shown to enhance virus replication and infectivity through a mechanism that involves enhanced biosynthesis and transportation of cholesterol

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and ganglioside to lipid rafts (Zheng et al., 2003). Lipid rafts are microdomains in the plasma membrane enriched in sphingolipids and cholesterol that are essential for egress from and entry of HIV into target cells (Zheng et al., 2001, 2003).

Comparison of SIV and HIV-1 Nef has revealed similarities and differences in function and structure between these proteins (Piguet and Trono, 1999). Both SIV and HIV-1 Nef show similar in vitro activities: down-regulation of CD4 and MHC-I cell surface expression, enhancement of virion infectivity, stimulation of viral replication in primary lymphocytes, modulation of T-cell signaling pathway and interaction with cellular serine/threonine and tyrosine kinases. The Nef proteins of both viruses contain a myristylated N-terminal and show homology, mainly in the central domain (Renkema and Saksela, 2000; Shugars et al., 1993). Despite these similarities, some noteworthy differences exist between SIV and HIV-1 Nef. In contrast to HIV-1 Nef, SIV-Nef contains additional amino acids residues at the N-terminus that are absent in HIV-1 Nef. Moreover, SIV and HIV-1 Nef interact with cellular proteins through distinct motifs and may utilize overlapping but distinct target sites for CD4 down-regulation (Bresnahan et al., 1998; Craig et al., 1998; Greenberg et al., 1998; Hua and Cullen, 1997; Lock et al., 1999; Piguet et al., 1998). HIV-1 Nef contains three repeats of the putative SH3 binding domain (PxP) important for the binding of Src family tyrosine kinases. In contrast, SIV-Nef contains only one corresponding PxP motif (Arold et al., 1997; Lee et al., 1995; Saksela et al., 1995). Thus, SIV-Nef and HIV-1 Nef are functionally similar but utilize different mechanisms to carry out similar functions (Bresnahan et al., 1998; Greenway et al., 1999; Hua and Cullen, 1997; Iafrate et al., 1997; Lang et al., 1997; Lock et al., 1999; Piguet et al., 1998).

SHIV nef chimeric viruses, constructed by replacing the SIVmac 239 *nef* gene with HIV-1 nef in a SIVmac 239 backbone, provided a valuable tool for investigating the interchangeability of SIV and HIV-1 Nef protein functions (Kirchhoff et al., 1999; Mandell et al., 1999; Shibata et al., 1991, 1997; Sinclair et al., 1997). These studies demonstrated that HIV-Nef expression in macaque cells exerted similar cellular effects and that HIV Nef and SIV-Nef are functionally interchangeable. Rhesus macaques experimentally infected with SHIV nef clones revealed that HIV Nef induces an AIDS-like disease in macaques. While the functional equivalence between HIV-1 and SIV-Nef has been demonstrated in vitro and in vivo, the suitability of the SHIV/macaque model for the study of the role of HIV-1 Nef in viral pathogenicity is yet to be determined.

Previous work investigating the mechanisms by which Nef exerts its effects on $CD4^+$ T cells has focused on delineating posttranscriptional interactions between Nef and cellular signaling and trafficking proteins (Piguet and Trono, 1999). However, effects of Nef on the transcription of genes involved in immune evasion and viral persistence have not been fully investigated. Given the complexity of pathways mediating these cellular processes and the paucity of information on the underlying mechanisms of Nef effects on T cells, we elected to characterize global gene expression profiles in Nef-expressing $CD4^+$ T cells using DNA microarray analysis. The effects of Nef on the transcription of cellular genes in different cell types of epithelial, neural and lymphocytes origin were previously investigated using DNA microarray analysis (Kramer-Hammerle et al., 2005; Shaheduzzaman et al., 2002; Simmons et al., 2001; van't Wout et al., 2005). These studies reported that Nef induced a transcription profile that enhanced cell activation and increased expression of genes that regulate apoptosis, biosynthesis and transport of membrane lipids. However, due to differences in cell type, the timing and the levels of Nef expressed, relevance of the processes in Nef-expressing cells during viral infection has been difficult to ascertain. We developed a Jurkat T-cell line that stably expresses SIV-Nef protein under the control of an inducible promoter (Ndolo et al., 2002). Human T-cell lines have been extensively used for the expression of SIV-Nef by other investigators and it was shown that SIV-Nef exerted similar effects as HIV-1 Nef expressed in human cells (Alexander et al., 1999; Baur et al., 1994; Sawai et al., 1995; Walk et al., 2001; Yoon et al., 2001). Similar Nef activities have also been reported in primary rhesus macaques CD4 T cells infected with SHIV nef recombinant viruses (Kirchhoff et al., 1999; Mandell et al., 1999; Shibata et al., 1997; Sinclair et al., 1997). We show that Nef mediates the down-modulation of the transcription of genes belonging to members of the MHC-I complex that mediate antigen presentation, thus facilitating immune evasion. Previous studies focused on endocytotic mechanisms. In addition, SIV-Nef enhances the transcription of genes encoding enzymes and factors that catalyze the biosynthesis of membrane lipids, facilitating the formation of lipid rafts that are pivotal for enhanced viral infectivity. Our data are in agreement with recently reported HIV Nef-mediated up-regulation in T cells of genes encoding enzymes involved in RNA processing, protein synthesis and cholesterol biosynthesis (Shaheduzzaman et al., 2002). Overall, our study indicates that SIV-Nef induces a transcriptional profile in $CD4^+$ T cells that promotes immune evasion and cell survival, thus facilitating viral persistence.

Results

Ecdysone-inducible expression of SIV-Nef

Previous attempts to stably express Nef in T cells have been hampered by the fact that long-term expression of Nef is toxic and results in selection of truncated Nef protein and eventual loss of expression (Baur et al., 1997; Baur et al., 1994; Walk et al., 2001). To overcome these limitations, we used the ecdysone-inducible expression system to achieve regulated expression of SIV-Nef in Jurkat T cells. A low basal level of Nef expression was observed in Jurkat T cells transfected with the pIND-CD8-nef and pVgRxR plasmids but in the absence of ponasterone A induction. Since leakage proof expression of recombinant proteins is difficult to achieve in inducible protein expression systems, we selected clone B5 which showed a five-fold increase in Nef expression upon induction but had a minimal basal expression in Nef-expressing Jurkat T cells in the absences of ponasterone A induction (Fig. 1). Expression levels of Nef protein at 24 h post-induction were found to be equivalent to levels of Nef

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