

## INTERVIEW about

# Immune evasion and the Nef protein of HIV-1 with

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### What is known about the Nef protein of HIV-1?

**Ashutosh Chaudhry & Satyajit Rath:** The *nef* gene is highly conserved in all primate lentiviruses (HIV-1, HIV-2 and SIV) and encodes a small protein of ~27 kDa that is expressed abundantly in the early stages of viral replication (about three quarters of the early viral mRNA load).

The Nef protein is post-translationally modified by phosphorylation and amino-terminal myristoylation, which allows it to target the cellular membranes. Nef contributes

to viral pathogenesis and disease progression as well as to viral infectivity, replication and dissemination (1). Nef inhibits death of HIV-1-infected host macrophages, creating a state of persistent infection (2). Nef also stimulates macrophages to initiate the attraction and activation of T cells which can then be infected (3). In addition, Nef downregulates a variety of cell surface molecules required for antigen presentation and thus contributes to immune evasion.

### How can Nef modulate the expression of cell surface proteins on dendritic cells?

**Ashutosh Chaudhry & Satyajit Rath:** Nef affects the expression of several cell surface proteins that play a role in the immune synapse. Nef downregulates cell surface MHC class I (MHCI) as well as class II (MHCII) (4-6). It also upregulates surface expression of the invariant chain (Ii, CD74) and DC-SIGN (CD209) (7-8). We and others have recently shown that Nef downregulates the cell surface costimulatory molecules CD80 and CD86 on macrophages and dendritic cells (9-10).

All these effects of Nef are post-transcriptional since total protein levels are unaffected. Thus, Nef alters the trafficking and distribution of these proteins. Lacking any enzymatic activity, Nef can be seen as an assembly of protein-protein interaction domains, and exerts its effects by bringing together effector

molecules that are part of normal cellular signaling or trafficking pathways.

Nef-mediated MHCII downregulation has been well studied and is thought to involve accelerated endocytosis of cell surface MHCII molecules into ARF-6-marked endosomes, followed by PACS-1-directed transit to trans-Golgi network (TGN) (4). MHCII molecules on the other hand are relocated to lysosomes in the presence of Nef, although it is still unclear whether they are internalized from the cell surface or simply retained in lysosomes while recycling (5,7). Nef-dependent upregulation of Ii occurs by PI-3-kinase-dependent pathways involving reduced trafficking of Ii to lysosomes for degradation (7). Nef mediates internalization of cell surface CD80 and CD86 and their relocation to the Golgi by a novel endocytic axis (unpublished data).

These effects of Nef on cell surface molecules are specific, since some proteins are downregulated while others are not. How is specificity maintained? The answers are

not clear yet. Nef directly binds to CD80 and CD86 (9), but its binding to other targets is either weak (MHCI; (11)), or not yet reported.

### Has the Nef-mediated loss of surface proteins any functional influence?

**Ashutosh Chaudhry & Satyajit Rath:** Nef-mediated downregulation of both HLA-A and HLA-B molecules can lead to poor presentation of viral epitopes on MHCI. This could lead to host CD8 T cells being unable to recognize and kill HIV-infected cells. HLA-C and HLA-E expression is not affected by Nef, and this could be protecting infected cells from NK cell-mediated lysis (12). Downmodulation of MHCII by Nef also results in reduced peptide presentation to CD4 T cells (5).

Although Nef mediates removal of MHC molecules, the strategy it uses for this still allows these molecules to reach

cell surface and function until their removal. Moreover, Nef does not affect the expression of all MHC isotypes, such as HLA-C, which can also mediate antigen presentation to some extent. In this context, it is interesting that Nef prevents primary T cell activation even under circumstances in which peptide-MHC complex presentation is not affected, probably as a result of the downregulation of cell surface costimulatory molecules such as CD80 and CD86 (9). Thus, Nef is likely to use multiple distinct mechanisms targeting cell surface proteins on dendritic cells to mediate effective immune evasion.

### Is there any possibility to modulate the influence of Nef in patients?

**Ashutosh Chaudhry & Satyajit Rath:** The multiple functions of Nef are clearly of interest for anti-HIV therapeutic strategies. Preventing Nef expression would lead to some impairment of viral replication as well as reduced infectivity and dissemination. However, these may not be major advantages, since *nef*-deficient HIV strains do grow in tissue culture. Blocking the interactions of Nef with various cellular proteins may lead to reduced pathogenesis in a variety of ways. Firstly, it may block the anti-apoptotic property of Nef, allowing infected cells to die faster. Blocking the Nef domains used in activating myeloid cells may prevent the recruitment of primed T cells as substrates for virus infection. Blocking the ability of Nef to mediate downregulation of MHC and costimulatory molecules may also allow far more efficient detection and killing of infected cells. Together, such Nef-directed interventions might reduce the

virus load in patients.

Recently, small molecular weight compounds that can successfully compete and block specific interactions of Nef have been described (13-14). Such compounds could provide candidate drugs to block HIV pathogenesis and AIDS progression.

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