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Hemopoietic cell kinase (Hck) and p21-activated kinase 2 (PAK2) are involved in the down-regulation of CD1a lipid antigen presentation by HIV-1 Nef in dendritic cells

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ABSTRACT

Dendritic cells (DCs) play a major role in *in vivo* pathogenesis of HIV-1 infection. Therefore, DCs may provide a promising strategy to control and eventually overcome the fatal infection. Especially, immature DCs express all CD1s, the non-MHC lipid antigen -presenting molecules, and HIV-1 Nef down-regulates CD1 expression besides MHC. Moreover, CD1d-restricted $CD4^+$ NKT cells are infected by HIV-1, reducing the number of these cells in HIV-1-infected individuals. To understand the exact role of DCs and CD1-mediated immune response during HIV-1 infection, Nef down-regulation of CD1a-restricted lipid/glycolipid Ag presentation in iDCs was analyzed. We demonstrated the involvement of the association of Nef with hemopoietic cell kinase (Hck) and p21-activated kinase 2 (PAK2), and that Hck, which is expressed strongly in iDCs, augmented this mutual interaction. Hck might be another therapeutic target to preserve the function of HIV-1 infected DCs, which are potential reservoirs of HIV-1 even after antiretroviral therapy.

Introduction

CD1 glycoproteins are non-classical MHC class I-like molecules that present lipid antigen (Ag) to CD1-restricted T cells. Although their role has been mainly studied in mycobacterial infections, several reports have suggested that CD1 molecules may also play important roles in viral infections (Chen et al., 2006; Moll et al., 2010; Raftery et al., 2008; Raftery et al., 2006; Shinya et al., 2004). CD1d-restricted NKT cells respond to infections by HIV-1 (Moll et al., 2009), HSV (Yuan et al., 2006), and influenza virus (De Santo et al., 2008). However, it is still not clear whether the T cell receptor (TCR) recognizes the viral antigens (Ag) presented by CD1 molecules, or the self-lipids induced by cellular metabolism due to viral infection, or a combination of both. It has been speculated that viral Ag could be presented by CD1c molecules as N-terminally acylated lipopeptides, similar in sequence to HIV-1 Nef (Van Rhijn et al., 2009). However, there is no evidence that this happens during viral infection.

The function and size of the human T cell repertoire that recognizes lipid Ag presented by CD1 molecules remains poorly defined. However a recent report showed that a large portion of circulating T cells appeared to be CD1a-autoreactive, and had all the known functional properties of Th22 cells including the expression of skin homing molecules (CCR4, CCR10, and CLA) (de Jong et al., 2010), suggesting the importance of CD1a-restricted T cells in skin immunosurveillance and possibly immunopathology. Moreover, a second, large population of circulating T cells, which remains to be characterized, appears to be restricted to CD1c and can recognize endogenous Ag (de Lalla et al., 2011). Importantly, viruses have evolved a series of mechanisms that directly interfere with the plasma-membrane expression of CD1 molecules, suggesting that CD1-restricted T cells may also participate in protection during viral infections. Indeed, we and others have reported that HIV-1 Nef protein down-regulates CD1a and CD1d surface expression in immature DCs (iDCs) (Cho et al., 2005; Shinya et al., 2004). This could lead to reduced Ag presentation, and represents an evasion mechanism of the pathogen similar to that responsible for the immune-evasion of HIV-1 infected T lymphocytes from cytotoxic T lymphocyte recognition following the down-regulation of peptide Ag presentation by MHC class I molecules (Collins et al., 1998). Of the accessory genes of HIV-1, *nef* is well known as a key factor of immune-evasion. In addition to down-regulating MHC class I, HIV-1 Nef also down-regulates MHC class II surface

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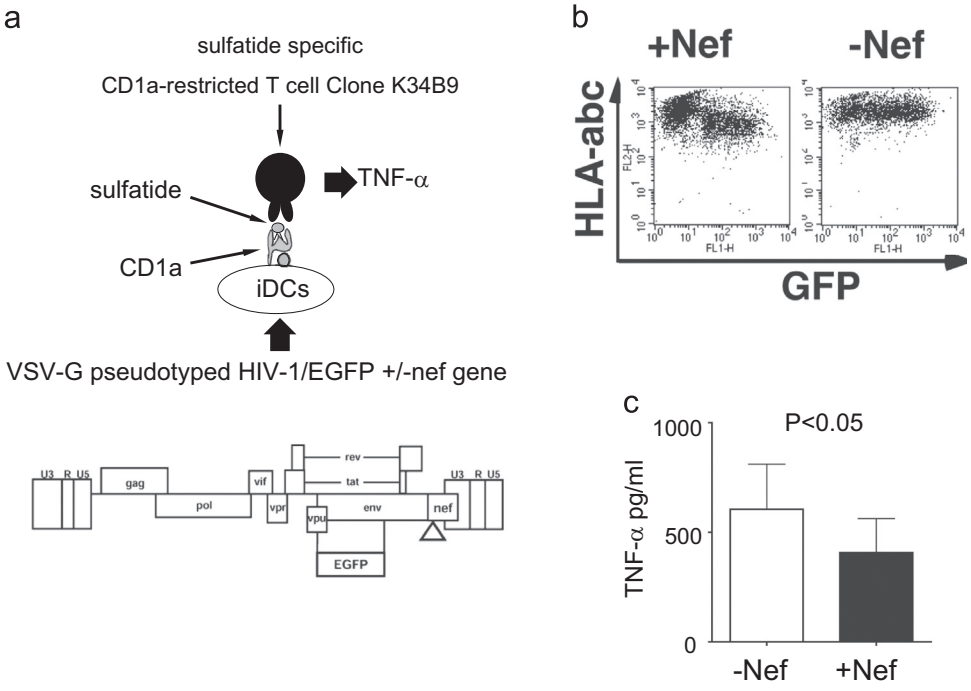

Fig. 1. Down-regulation of CD1a lipid antigen presentation on iDCs infected with VSV-G-pseudotyped recombinant HIV. a) The CD1a lipid antigen presentation assay. PBMC-derived iDCs were used as antigen presenting cells and infected with VSV-G pseudotyped single-cycle recombinant HIV-1/EGFP (with the intact *nef* gene designated as +Nef or with the crippled *nef* gene designated as -Nef). Subsequently, the DCs were incubated with sulfatide to stimulate the T cell clone K34B9.1 and TNF-α released in the supernatants was measured by ELISA. b) and c) The infection of iDCs by VSV-G-pseudotyped HIV-1 with the intact *nef* gene (+Nef) showed no more than 30% of infected (EGFP positive) iDCs, which still showed a significant reduction of TNF-α production compared to the crippled *nef* gene (-Nef) ($P < 0.05$ by the paired *t* test).

expression (Stumptner-Cuvelette et al., 2001). Furthermore, it has recently been reported that HIV-1 Vpu together with Nef inhibits lipid Ag presentation in DCs by CD1d (Moll et al., 2010). However, with regards to CD1a, only the down-regulation of surface expression of CD1a in iDCs has been reported (Shinya et al., 2004).

Myeloid iDCs are the only peripheral Ag presenting cells (APCs) that are known to express all human CD1 isoforms (CD1a, CD1b, CD1c, CD1d, and CD1e) and initiate lipid Ag processing pathways in response to activating stimuli. Moreover, $CD1a^+$ iDCs, or Langerhans cells, are thought to be the first cells to encounter HIV-1 at mucous membranes, and capture viral particles to allow them productive replication and long-term viral dissemination that are later transferred to $CD4^+$ lymphocytes (Burleigh et al., 2006; Coleman et al., 2011; Dong et al., 2007; Turville et al., 2004; Wang et al., 2007). On the other hand, the C-type lectin DC-SIGN is expressed on the surface of iDCs and enhanced HIV-1 trans-infection (Geijtenbeek et al., 2000). DC-SIGN positive iDCs from human rectal mucosa is known to bind and transfer HIV-1 to CD4+ T cells efficiently and, in human rectal mucosa, DC-SIGN antibodies could block 90% of HIV-1 binding although only 1–5% of total mucosal mononuclear cells (Gurney et al., 2005). Taken together, iDCs seem to be more relevant in establishing an immune response against HIV than mature DCs.

In this study, we used PBMC-derived iDCs to show that HIV-1 Nef significantly down-regulated lipid Ag presentation by CD1a together with its surface expression on iDCs. Furthermore, using a series of mutant *nef* genes, we confirmed the intermolecular interaction of HIV-1 Nef and CD1a together with hemopoietic cell kinase (Hck) and p21-Activated Kinase 2 (PAK2). Hck was highly expressed in iDCs and HIV-1 takes advantage of Hck in iDCs as well as PAK2 for the down-regulation of CD1a lipid Ag presentation and immune-evasion from this lipid Ag recognition system.

Results

CD1a lipid Ag presentation is impaired by Nef in HIV-1 infected iDCs

In this study, we analyzed the influence of HIV-1 Nef on CD1a lipid Ag presentation. As antigen presenting cells (APCs), we used peripheral blood mononuclear cells (PBMC)-derived iDCs to measure CD1a lipid Ag presentation, (Figs. 1 and 2). Since iDCs are resistant to transfection by conventional techniques such as those used with DNA plasmids, we used the VSV-G pseudo-typed single-cycle recombinant HIV-1 vector (Shinya et al., 2003, 2004) to introduce the *nef* gene into iDCs (Fig. 1a). The CD1a-restricted T cell clone K34B9.1, which is both CD1a restricted and sulfatide-specific, was used as a responder cell (Shamshiev et al., 2002).

Despite an efficiency of infection no greater than 30% (Shinya et al., 2004), on infection of PBMC-derived iDCs with the single-cycle reporter HIV-1 pseudotyped with VSV-G (Fig. 1b), there was significant down-regulation of TNF-α secretion by the virus encoding the *nef* gene (+Nef) relative to the virus not expressing Nef due to the *crippled nef* gene (−Nef, Fig. 1c), suggesting that HIV-1 Nef abrogated CD1a lipid Ag presentation.

To obtain higher efficiency expression, *EGFP* mRNA was electroporated into iDCs (Fig. 2a) and showed greater than 90% GFP+ expression in the iDCs (Fig. 2b). Moreover, the mRNA electroporation with the *EGFP* gene did not cause the significant changes in the surface expression of CD1a, HLA-abc, CD83 or DC-SIGN (Fig. 2b).

Mutation in the PXXP SH3 binding motif and R106 abrogated the Nef-mediated impairment of CD1a lipid Ag presentation

A series of mutations were introduced into the *nef* gene (Fig. 3), which was fused in frame to the 5′ end of the *EGFP* gene and the mRNA of the *nef -EGFP* gene capped with the anti-reverse cap