**ABSTRACT**

Plasmacytoid dendritic cells (pDCs) are the major source of type I IFN (IFN-α) in response to HIV-1 infection. pDCs are rapidly activated by HIV-1 infection and are implicated in both early reduction of viral load and HIV-1-induced pathogenesis. However, the mechanism of HIV-1 recognition by pDCs and pDCs activation is not clearly defined. In this study we showed that two similar HIV-1 variants (R3A and R3B) isolated from a rapid progressor differentially activate pDCs to produce IFN-α. The highly pathogenic R3A induced robust IFN-α production in pDCs, while the less pathogenic R3B did not. The viral determinant of efficient IFN-α induction was mapped to R3A Env and its V1V2 region with enhanced CD4 binding activity. Furthermore we showed that the Nef protein was also required for the IFN-α induction from pDCs. Analysis of a panel of R3A Nef functional mutants indicated that Nef domains involved in CD4 down-regulation were necessary for R3A to induce IFN-α in pDCs. Our data indicate that R3A-induced pDCs activation depends on (1) the high affinity of envelope to bind CD4 receptor and (2) the Nef activity involved in CD4 down-regulation. Our work provides new insights into the mechanism by which HIV-1 stimulates IFN-α in pDCs and contributes to the pathogenesis of HIV-1 infection.

**RESULTS**

1. A highly pathogenic HIV-1 isolate R3A induces robust IFN-α production in a Nef-dependent fashion. Total human PBMC, pDC-depleted PBMC and purified pDCs were stimulated with HIV-1 isolates R3A, R3B and their Nef mutants (R3A-ΔNef and R3B-ΔNef). IFN-α was measured by Elisa at 18-20h after stimulation.

2. pDCs are the major source of IFN-α after stimulation with HIV-1 R3A isolate. Total PBMC and pDC-depleted PBMC were stimulated with R3A, R3B, R3A-ΔNef and R3B-ΔNef. IFN-α was measured by Elisa at 96h after stimulation.

3. CD4 binding, membrane fusion and endocytosis are required for R3A-mediated pDC activation. (A) PBMC were stimulated with R3A in the presence of inhibitors of CD4-binding: soluble CD4 and anti-gp120 neutralizing antibodies (gp120/nAb), fusion inhibitor T20 and inhibitors of endocytosis: chloroquine (Chlor) and bafilomycin A1 (BafA). (B) Purified pDCs were stimulated with R3A in the presence of fusion inhibitor T20 and reverse transcriptase inhibitor Nevirapine (RTi inhib). IFN-α was measured as above.

4. V1V2 region of R3A Env with high CD4 binding affinity determines the pDCs activation by R3A. PBMCs were stimulated with R3A, R3B and their recombinants: R3A with R3B V1V2 (R3A/BV1V2) and R3B with R3A V1V2 (R3B/A V1V2). IFN-α was measured as above.

5. The CD4 down-modulation activity of Nef is required for R3A-mediated IFN-α induction from pDCs. (A) The schematic representation of Nef and AIA substitution mutations (bold and underlined) affecting specific Nef activities (Table 1). (B) The Nef motifs required for CD4 down-regulation are also required for IFN-α induction. The nef- mutated viruses and control R3A and R3A-ΔNef viruses were used to stimulate human PBMC. IFN-α was measured as above.

**CONCLUSIONS**

1. Highly pathogenic phenotype of HIV-1-R3A correlates with enhanced ability of this strain to activate pDCs and induce IFN-α.

2. Env and Nef protein are the viral determinants of the robust IFN-α induction in pDCs.

3. Both, R3A Env-V1V2 and its up-regulation by Nef-dependent CD4-down-modulation, lead to increased CD4 binding and endocytosis of R3A into pDCs.

This work was supported by NIH grants AI080432 and AI095097.

**Table 1. Comparison of motif-dependent specific biological activities of Nef with motif-dependent ability to activate pDCs analyzed in this study.**

<table>
<thead>
<tr>
<th>Nef Mutations</th>
<th>Function, interaction with host protein</th>
<th>CD4 down-regulation</th>
<th>IFN-α induction</th>
<th>pDC stimulation</th>
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<tr>
<td>(+) functional (+/−) partial loss of function (−) loss of function (−/−) not determined; (n) not required for function</td>
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1 As numbers are indicated after last residues in the motif
2 Infectivity (assay on MagiX4)

**Figure Legend**

- **Figure 1.** Illustration of the interaction between HIV-1 and Nef proteins with host cell receptors.
- **Figure 2.** Diagram showing the expression levels of Nef proteins in different cell types.
- **Figure 3.** Flowchart depicting the activation of pDCs by HIV-1 variants.
- **Figure 4.** Graphs illustrating the effects of Nef mutants on CD4 down-regulation and IFN-α induction.
- **Figure 5.** Bar charts comparing the biological activities of different Nef variants.