HIV-1 Env and Nef cooperatively contribute to pDCs activation via CD4-dependent mechanisms

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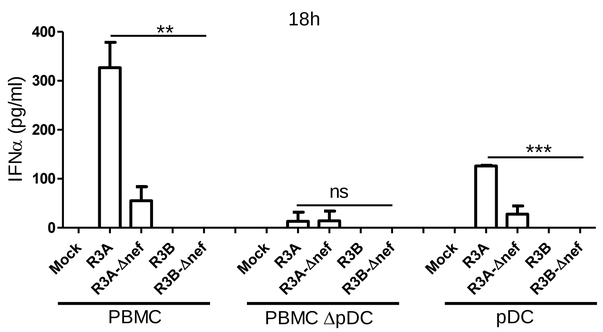
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ABSTRACT

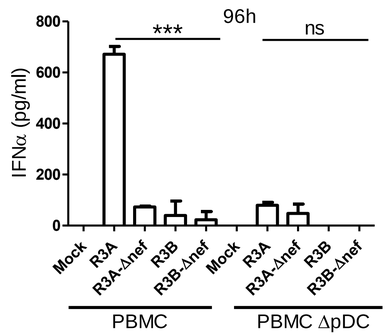
Plasmacytoid dendritic cells (pDCs) are the major source of type I IFN (IFN-I) in response to HIV-1 infection. pDCs are rapidly activated by HIV-1 infection and are implicated in both early reduction of viral load and HIV-1 induced pathogenesis. However, the mechanism of HIV-1 recognition by pDCs and 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 IFNα. The highly pathogenic R3A induced robust IFNα production in pDCs, while the less pathogenic R3B did not. The viral determinant of efficient IFNα 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 IFNα 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 IFNα in pDCs. Our data indicate that R3A- induced pDCs activation depends on (1) the high affinity of envelop 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 IFNα in pDCs and contributes to the pathogenesis of HIV-1 infection.

RESULTS

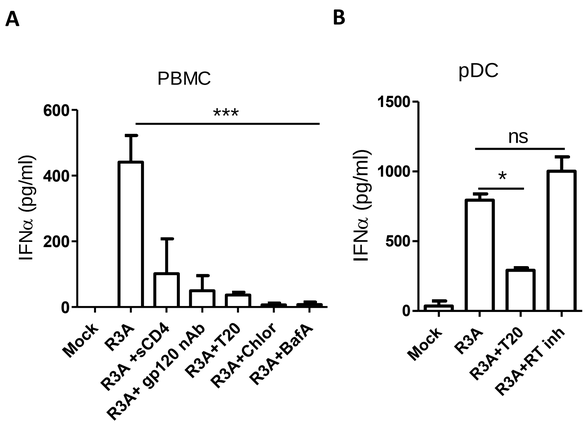
1. A highly pathogenic HIV-1 isolate R3A induces robust IFNα production in a Nef-dependent fashion. Total human PBMC, pDC-depleted PBMC and purified pDCs were stimulated with HIV-1 isolates R3A, R3B and their Nef mutants (R3A-Δnef and R3B-Δnef). IFNα was measured by Elisa at 18-20h after stimulation.



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

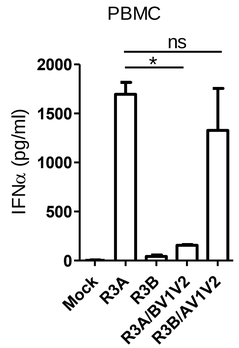


3. CD4 binding, membrane fusion and endocytosis are required for R3A –mediated pDC activation. (A) PMBC were stimulated with R3A in the presence of inhibitors of CD4-binding: soluble CD4 and anti-gp120 neutralizing antibodies (gp120nAb), fusion inhibitor T20 and inhibitors of endocytosis: chloroquine (Chlor) and bafilomycin A1 (BafA). (B) Purified pDCs were stimulated with R3A in the presence of fusion inhibitor T20 and reverse transcriptase inhibitor Nevirapine (RT inh). IFNα was measured as above.

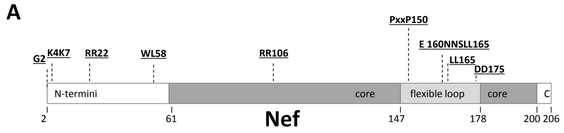


* p≤0.05 , **p≤0.01; ***p≤0.001, ns - not significant.

4. V1V2 region of R3A Env with high CD4 binding affinity determinates the PBMC/pDC 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. IFNα was measured as above.



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



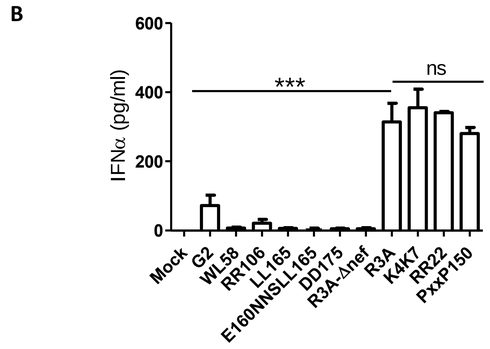


Table 1. Comparison of motif-dependent specific biological activities of Nef with motif-dependent ability to activate pDCs analyzed in this study.

Residues [1]	Function, interaction with host protein	REF	Results of our study	
		CD4↓	Infectivity [2]	pDC stimulation
G2	N-terminal myristoylation	-	-	-
K4K7	lipids rafts targeting	?	+	+
RR22	membrane targeting	n	+	+
WL58	CD4 /viral protease cleavage site	-	-	-
RR106	Pak 2 CD4 /salt bridges formation, oligomeryzation	+/-	-	-
PxxP150	SH3 domains	n	+	+
LL165	clathrin- adaptor protein AP2	-	-	-
E160NNSLL165	clathrin- adaptor proteins AP1, AP2, AP3	-	-	-
DD175	clathrin- adaptor protein AP-2 and V1H of ATPase	-	-	-

[1] Aa numbers are indicated after last residues in the motif
[2] Infectivity (assay on MagiX4)
(+) functional (+/-) partial loss of function (-) loss of function
(?) -not determined; (n)-not required for function

CONCLUSIONS

1. **Highly pathogenic phenotype of HIV-1-R3A correlates with enhanced ability of this strain to activate pDCs and induce IFNα**
2. **Env and Nef protein are the viral determinants of the robust IFNα 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**

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