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Human Immunodeficiency Virus Type 1 gp120 and Other Activation Stimuli Are Highly Effective in Triggering Alpha Interferon and CC Chemokine Production in Circulating Plasmacytoid but Not Myeloid Dendritic Cells

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Exposure to aldrithiol-2-inactivated human immunodeficiency virus type 1 or gp120, but not gp41, triggered alpha interferon (IFN-α), CC chemokine ligand 2 (CCL2), CCL3, and CCL4 production in human plasmacytoid dendritic cells (DCs) but not in myeloid DCs (M-DCs) or monocyte-derived DCs from the same donors. The nonresponsiveness of M-DCs for IFN-α/β production was a general feature specific to these cells, as they also failed to produce it in response to inactivated influenza virus, poly(I-C), lipopolysaccharide, *Staphylococcus aureus* Cowans I, or CD40L. The different capacities of circulating DC subsets to produce immune mediators in response to most stimuli argue for a different role for these cells in the regulation of innate immunity to pathogens.

Dendritic cells (DCs) are a heterogeneous population of antigen-presenting cells controlling the initiation of T-cell-dependent immune responses (16, 26). Two distinct DC subsets, defined as myeloid DCs (M-DCs) and plasmacytoid DCs (P-DCs), have been identified in human blood (1, 4, 20). P-DCs produce large amounts of alpha/beta interferon (IFN-α/β) in response to viruses and other pathogens (2, 17), whereas M-DCs are the main interleukin-12 (IL-12) producers (1, 24). Likewise, P-DCs and M-DCs produce different patterns of chemokines and exhibit different migratory responses upon exposure to a variety of stimuli/ligands (22, 23).

Early interactions between viruses and DCs have recently gained considerable interest for their possible importance in the pathogenesis of viral infections (21). In human immunodeficiency virus type 1 (HIV-1) infection, DCs play a key role both in the induction of antiviral immune responses and in the viral transmission to $CD4^+$ T cells (18). DCs are susceptible to HIV infection and represent one of the earliest cell types exposed to the virus in vivo (14, 18). As a key cell type in innate antiviral immunity, P-DCs have been studied in more detail in the setting of HIV-1 infection and characterized for their capacity to directly recognize and respond to HIV-1 infection by producing large quantities of IFN-α (8, 9, 27). Virus-induced IFN-α contributes, at least in part, to the restriction of viral replication in P-DCs (8, 27) and to P-DC-induced M-DC maturation (9). Despite these observations, the specific HIV-1 components involved in this phenomenon are poorly characterized. Moreover, comparative studies evaluating whether circulating DC subsets and in vitro-generated monocyte-derived DCs (MD-DCs) from the same donor differentially respond to viral, bacterial, and T-cell-derived stimuli by producing immune mediators have not yet been performed.

We therefore compared the capacities of human circulating P-DCs and M-DCs and in vitro-generated MD-DCs to produce IFN-α upon early HIV-1 interaction. P-DCs and M-DCs were magnetically sorted from peripheral blood mononuclear cells with BDCA-4 and BDCA-1 cell isolation kits (Miltenyi Biotec, Bergish Gladbach, Germany), respectively, as described previously (22), whereas MD-DCs were obtained from monocytes isolated by negative immunoselection of peripheral blood mononuclear cells from the same donor. Blood DC subsets were phenotypically defined by flow cytometry as lacking lineage markers and expressing CD123 and BDCA-2 (P-DCs) or CD11c and BDCA-1 (M-DCs). Monoclonal antibody (MAb) antilineage mixture 1 fluorescein isothiocyanate (FITC) (containing MAbs specific for CD3, CD14, CD16, CD19, CD20, and CD56), anti-CD123 phycoerythrin (PE), anti-CD11c PE, and the respective FITC- or PE-conjugated isotype control MAbs were from BD Pharmingen (San Diego, CA). MAbs anti-BDCA-2 FITC and anti-CD1c (BDCA-1) PE were from Miltenyi Biotec. Cells were then cultured in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) containing 5 to 10% fetal bovine serum plus 50 ng/ml granulocyte-macrophage colony-stimulating factor and 500 U/ml IL-4 (Schering-Plough, Dardilly, France; M-DCs and MD-DCs) or 10 ng/ml IL-3 (BD Pharmingen; P-DCs). Blood DC subsets were exposed to aldrithiol-2 (AT-2)-inactivated HIV-1 (25) at the time of seeding, while MD-DCs were exposed after 6 days of culture. IFN-α secretion in culture supernatants was assessed 24 h later. As shown in Fig. 1A, P-DCs, but not M-DCs or MD-DCs, produced high levels of IFN-α upon exposure to

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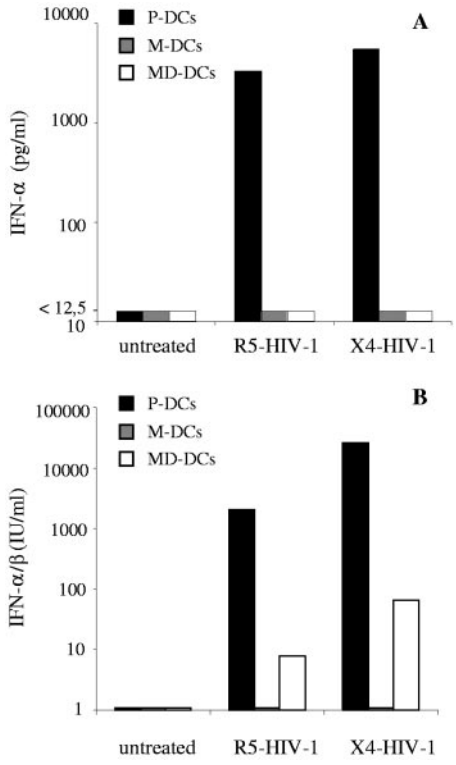

FIG. 1. IFN-α/β production by circulating DC subsets and in vitro-generated myeloid DCs exposed to AT-2-inactivated X4 and R5 HIV-1 strains. (A) P-DCs (2.5×10^5/ml), M-DCs (2.5×10^5/ml), and MD-DCs (1×10^6/ml) were cultured in a 96-well, flat-bottomed tissue culture plate in 5 to 10% fetal bovine serum-RPMI 1640 medium and exposed to X4 (HIV-1_{IIIB}) and R5 (HIV-1_{BaL}) AT-2-inactivated HIV strains (dose equivalent to a multiplicity of infection of 0.1 before inactivation). Twenty-four hours later, culture supernatants were collected and the IFN-α content was measured by enzyme-linked immunosorbent assay (ELISA) (Pierce Endogen, Rockford, IL; detection limit, 12.5 pg/ml). A representative experiment out of six performed is shown. (B) The content of biologically active IFN-α/β was determined in the supernatants collected from the same cultures described above by a vesicular stomatitis virus cytopathic reduction assay. A representative experiment out of three performed is shown.

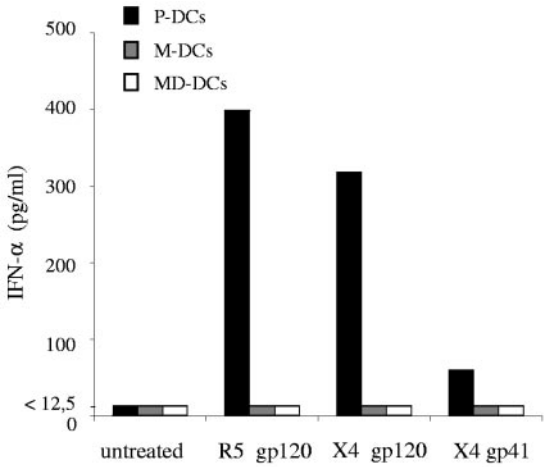

FIG. 2. Effect of recombinant gp120 and gp41 on the production of IFN-α by P-DCs, M-DCs, and MD-DCs. DC subsets were treated with 2 μg/ml of recombinant X4 or R5 HIV-1 gp120 (HIV-1_{IIIB} and HIV-1_{BaL}, obtained from the EU program EVA/MRC and the National Institutes of Health AIDS Research and Reference Reagent Program, respectively) or X4 gp41 (HIV-1_{IIIB}, purchased from ABI, Columbia, MD) for 24 h. Cell supernatants were collected, and the IFN-α content was measured by ELISA. A representative experiment out of three performed is shown.

AT-2 HIV-1 independently of the viral strain. To exclude the possibility that myeloid DCs could respond to HIV by producing other IFN subtypes, cell supernatants were analyzed for the presence of biologically active IFN-α/β by measuring their capacity to induce antiviral responses to vesicular stomatitis virus in HeLa cells (11). As shown in Fig. 1B, small amounts of IFN-α/β were indeed produced by MD-DCs in response to inactivated HIV-1, whereas no detectable IFN-α/β was found in M-DC supernatants. A remarkably higher IFN production was detected in P-DCs under the same experimental conditions.

To establish the relative importance of HIV-1 surface components in triggering IFN production, DC subsets were exposed to either recombinant gp120 or gp41, and IFN-α production was assessed 24 h later. As shown in Fig. 2, both X4 and R5 gp120 were per se sufficient to induce clear-cut IFN-α secretion in P-DCs, although to a lesser extent than the whole inactivated virus, while a negligible IFN-α secretion was observed upon gp41 treatment. Conversely, neither gp120 nor gp41 induced any IFN-α secretion in either M-DCs or MD-DCs (Fig. 2).

To further evaluate whether the inability of M-DCs and MD-DCs to produce IFN in response to HIV-1 was specific for this host-virus interaction or represented a broader incapacity of myeloid DCs to respond to IFN-inducing stimuli, P-DCs, M-DCs, and MD-DCs were exposed to a variety of stimuli of viral, bacterial, or T-cell origin. As shown in Fig. 3A, P-DCs produced large amounts of IFN-α in response to influenza virus and CD40L, exhibited a modest response to *Staphylococcus aureus* Cowans I (SAC), and as expected, failed to respond to poly(I-C) and lipopolysaccharide (LPS). Conversely, no IFN-α production was detected in either M-DCs or MD-DCs in response to any of the stimuli used. However, when the same supernatants were analyzed for the presence of biologically active IFN, a significant production was observed in MD-DCs stimulated with influenza virus, poly(I-C), LPS, and CD40L, but not with SAC, although these levels were lower than in P-DCs (Fig. 3B). In contrast, no IFN activity was found in M-DC cultures under any experimental condition tested (Fig. 3B). Under the same experimental conditions, M-DCs, but not P-DCs, selectively produced high levels of IL-10 in response to CD40L stimulation (data not shown), showing an additional level of diversity between human blood DC subsets.

We have previously reported that exposure of human peripheral blood macrophages to X4- and R5-inactivated HIV-1 strains or recombinant gp120 induces clear-cut up-regulation of CC chemokine secretion (5, 6) without significantly modifying chemokine production by MD-DCs (7). Moreover, we have shown that differential chemokine production in response to bacterial, viral, and T-cell-derived stimuli can functionally distinguish M-DCs and P-DCs (21). We therefore analyzed CC chemokine secretion in blood DC subsets upon HIV-1 exposure. As shown in Table 1, P-DCs responded to both X4- and

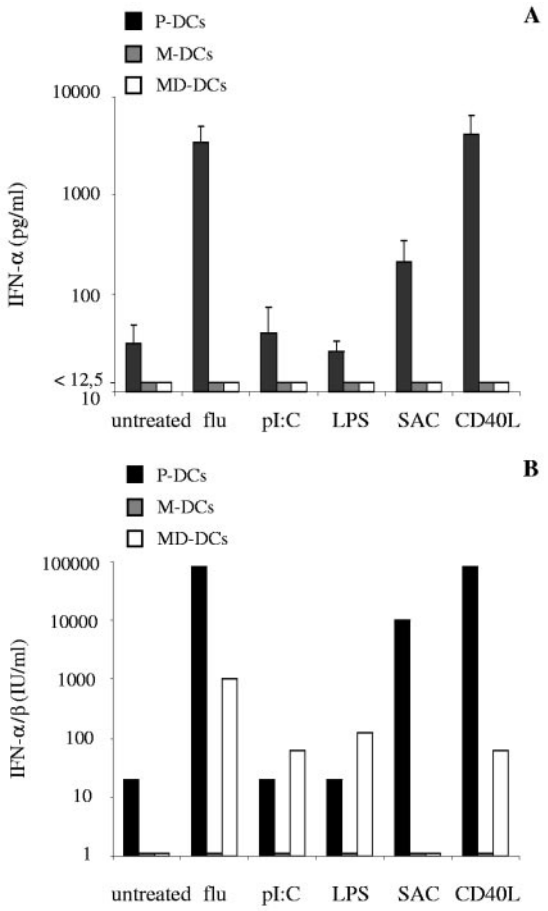

FIG. 3. IFN-α/β production by P-DCs, M-DCs, and MD-DCs in response to different IFN-inducing stimuli. (A) DC subsets were stimulated with inactivated influenza virus (strain A/Moscow/10/99, 20 ng/ml of hemagglutinin), poly(I-C) (pI:C; 20 μg/ml; Sigma-Aldrich, St. Louis, MO), LPS (100 ng/ml; Sigma-Aldrich), SAC (1:5,000; Calbiochem, San Diego, CA), and CD40L-transfected J558 cells at a ratio of 5:1 (23) or left untreated. Cell supernatants were collected 24 h later, and the content of IFN-α was determined by ELISA. The mean values ± standard deviations of six independent experiments are shown. (B) The content of biologically active IFN-α/β was determined in the supernatants collected from the same cultures as described in the legend to Fig. 1. A representative experiment out of three performed is shown.

R5-inactivated HIV-1 strains and gp120, as well as to CD40L stimulation, by secreting significant levels of CC chemokine ligand 2 (CCL2), CCL3, and CCL4. In contrast, M-DCs did not show any detectable change in the level of secreted chemokines upon gp120 stimulation, whereas a clear-cut increase in the secretion of CCL3 and CCL4, but not of CCL2, was observed following CD40 ligation (Table 1).

In keeping with the important role of P-DCs as the first line of defense against infectious pathogens, we report for the first time that their exposure to inactivated HIV-1 or gp120 is per se sufficient to trigger IFN-α and CC chemokine production. Previous in vivo studies have shown that IFN-α, produced by thymic P-DCs as a direct consequence of HIV infection, has a functional role in the regulation of major histocompatibility complex class I expression (13) and the induction of the antiviral state in thymocytes (12), indicating that the production of IFN-α and chemokines by blood P-DCs exposed to HIV could be functionally relevant in vivo. We also report that AT-2-inactivated viruses represent a more potent stimulus for IFN-α production than gp120 alone, suggesting that gp41, although per se ineffective, may enhance the gp120 effect. Our results are consistent with previous studies demonstrating that productive HIV-1 infection is not required for triggering IFN-α production in P-DCs (8, 27). In contrast, neither inactivated virus nor recombinant gp120 and gp41 induced any IFN-α production in M-DCs or MD-DCs.

It is widely recognized that the expression of IFN-α/β genes is a transient response (regulated at the transcriptional level) of most cell types to viruses or other IFN inducers (15). The nature of the IFN produced depends on both the cell type and the inducing agent. In this regard, it is noteworthy that circulating M-DCs are apparently refractory to the production of any IFN-α/β in response to any stimulus tested. Conversely, MD-DCs fail to produce IFN-α upon exposure to HIV-1 as well as to a variety of stimuli, including LPS, poly(I-C), influenza virus, SAC, and CD40L, but are fully competent to secrete biologically active IFN-β. In response to LPS and poly(I-C), the IFN-α/β secreted by MD-DCs has been characterized to be predominantly IFN-β (3, 10). Although MD-DCs are used largely as a model of the myeloid lineage and have been instrumental in defining many of the biologic paradigms of DC function, it is worth pointing out that MD-DCs and blood M-DCs exhibit qualitatively different responses in terms of IFN-α/β production.

Productively HIV-1_{BaL}-infected P-DCs can produce CCL3 and CCL5 (8). However, we report for the first time that inactivated HIV-1 or gp120 from X4 and R5 HIV strains selectively induces CC chemokine secretion in P-DCs but not in M-DCs. Interestingly, in contrast to what occurs with inactivated influenza virus (23), a remarkable secretion of CCL2 can be detected upon P-DC exposure to gp120. These results suggest that soluble mediators, produced by both infected and/or bystander uninfected P-DCs exposed to gp120, may represent a protective response to HIV infection and contribute to limit viral spreading by blocking either specific coreceptor usage (CC chemokines) or viral replication (IFN-α) in uninfected cells. These mediators may regulate both the course of HIV infection and the immune response to the virus by acting at different levels. The production of chemokines may represent a protective response of P-DCs to HIV infection and contribute to limiting viral spreading by blocking coreceptor usage in noninfected cells. Likewise, these factors may contribute to the immune response to HIV by recruiting specific immune cell populations. Conversely, hyperproduction of chemokines during the course of infection may enhance viral spreading by favoring the infection of newly recruited, susceptible immune cells. In this regard, it has been reported that herpes simplex virus-induced chemokine production in P-DCs plays an important role in the homing of leukocytes to these cells (19). Although the in vivo relevance for the enhanced chemokine production remains to be elucidated, it is likely that the balance of their negative-versus-positive effects on HIV infection may contribute to different outcomes of HIV disease.

Overall, these results indicate that DC subsets exhibit a distinct response to HIV-1 by producing a variety of immune

TABLE 1. Effect of recombinant gp120 and AT-2-inactivated HIV-1 X4 and R5 strains on the production of CC chemokines in P-DCs and M-DCs[a]

Cell type	Treatment	CC chemokine production (pg/ml)								
		CCL2			CCL3			CCL4		
		Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3
P-DCs	None	115	8	41	297	134	132	500	140	52
	X4-gp120	400	47	204	2,413	296	454	3,700	221	81
	X4–AT-2–HIV-1	400	129	238	3,783	373	644	4,196	235	410
	BaL-gp120	375	255	428	400	640	1,548	1,092	372	224
	BaL–A-T2–HIV-1	400	97	231	715	324	638	1,792	220	158
	CD40L	400	154	327	1,005	755	3,910	2,877	757	2,000
M-DCs	None	5	31	31	268	10	111	173	33	23
	X4-gp120	6	31	34	210	10	129	144	25	25
	X4–AT-2–HIV-1	6	31	8	210	10	99	250	24	21
	R5-gp120	8	31	5	122	10	177	85	21	23
	R5–AT-2–HIV-1	7	31	9	239	14	114	114	20	44
	CD40L	6	31	12	381	282	1,123	303	268	162

[a] DC subsets were treated with AT-2-inactivated HIV-1 (dose equivalent to a multiplicity of infection of 0.1 before inactivation) or recombinant gp120 (2 μg/ml) from X4 and R5 HIV-1 strains or CD40L-transfected J558 cells or were left untreated. After 24 h of culture, supernatants were harvested and frozen before CCL2, CCL3, and CCL4 determination by ELISA (R&D Systems, Minneapolis, MN; detection limits, 5 pg/ml, 10 pg/ml, and 4 pg/ml, respectively). The results obtained with dendritic cells isolated from three different donors are shown.

mediators. The vigorous response of P-DCs to early interactions with HIV surface components further highlights their role in the innate response to HIV-1 infection and, ultimately, in the pathogenesis of AIDS.

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