HIV-1-Induced Impairment of Dendritic Cell Cross Talk with \( \gamma \delta \) T Lymphocytes

Marco Cardone,* Kyojiro N. Ikeda,* Barbara Varano, Sandra Gessani, Lucia Conti

Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy

ABSTRACT

The interplay between dendritic cells (DC) and \( \gamma \delta \) T lymphocytes represents a network of paracrine and cell contact interactions important for an integrated immune response to pathogens. HIV-1 infection dramatically affects the number and functions of both cell populations, and DC/\( \gamma \delta \) T cell cross talk may represent a target of virus-induced immune escape. We investigated whether HIV-exposed DC could deliver aberrant signals to interacting \( \gamma \delta \) T cells. Here we report that the interaction of human \( \gamma \delta \) T lymphocytes with HIV-1-exposed autologous monocyte-derived DC, but not direct exposure to the virus, impairs lymphocyte expansion and gamma interferon (IFN-\( \gamma \)) production in response to phosphoantigens. This effect is independent of virus strain and occurred in 55% of the donors analyzed. The donor-dependent variation observed relies on the responsiveness of DC to HIV-1 and is strictly related to the capacity of the virus to suppress the maturation-induced expression of interleukin 12 (IL-12). In fact, \( \gamma \delta \) T cell response to phosphoantigens is almost completely recovered when this cytokine is exogenously added to the DC/lymphocyte cocultures. Interestingly, we show that \( \gamma \delta \) T lymphocytes are recruited by HIV-1-exposed DC through a CCR5-mediated mechanism and exert a CCL4-mediated control on virus dissemination within DC and susceptible CD4\(^+\) T lymphocytes. These results demonstrate an association between HIV-induced DC dysfunction and alterations of \( \gamma \delta \) T cell responses. The aberrant cross talk between these two cell populations may contribute to the pathogenesis of HIV infection by further reducing the strength of antiviral immune response.

IMPORTANCE

This study provides new evidence on the mechanisms exploited by HIV-1 to evade the host immune response. We report that HIV-1 impairs the cross talk between DC and \( \gamma \delta \) T lymphocytes, by reducing the capacity of DC to promote functional \( \gamma \delta \) T cell activation. Interestingly, the virus does not per se interfere with \( \gamma \delta \) T cell activation, thus highlighting the key role of early DC-HIV-1 interaction in this phenomenon. Furthermore, the results obtained unravel the novel role of \( \gamma \delta \) T cells in controlling HIV-1 dissemination within the DC population as well as virus transfer to susceptible CD4\(^+\) T lymphocytes. The interactions of DC with innate lymphocytes represent a major control mechanism for an integrated immune response to infection. Understanding how HIV-1 harnesses these pathways may provide important insights on the pathogenesis of disease and offer new opportunities for therapeutic interventions.

Human \( \gamma \delta \) T cells represent about 1 to 10% of peripheral blood CD3\(^+\) cells. In particular, cells expressing the V\( \gamma \)9V\( \delta \)2 T cell receptor (TCR) constitute the major population of circulating \( \gamma \delta \) T lymphocytes and are uniquely found in humans and primates. This subset responds to both pathogen- and host-derived small nonpeptide phosphorylated antigens and exert strong antimicrobial and antitumor activities (1, 2).

Alterations of blood \( \gamma \delta \) T cell distribution in human immunodeficiency virus (HIV)-infected individuals have been reported previously (3). Both a decrease in V\( \gamma \)9V\( \delta \)2 T cell count and impaired \( \gamma \delta \) T cell-mediated cytokine production have been described at early stages of infection (4, 5). Suppression of HIV replication by highly active antiretroviral therapy (HAART) was associated with no or slow recovery of both blood and mucosal V\( \gamma \)9V\( \delta \)2 T cell number and function (6–8). Moreover, the reactivity of V\( \gamma \)9V\( \delta \)2 T cells to Mycobacterium tuberculosis stimulation was drastically decreased or absent in a high proportion of HIV-infected individuals at late stages of disease (9). On the other hand, natural viral suppressors have been shown to exhibit frequencies of effector \( \gamma \delta \) T cells similar to those of non-HIV-infected individuals (10). Similarly, V\( \delta \)2 T cells from the simian immunodeficiency virus (SIV) natural hosts sooty mangabeys are not depleted and exhibit a normal activation potential and Th1 profile (11). Recently, a study by Li and colleagues correlated quantitative and qualitative abnormalities in V\( \delta \)2 T cells with HIV disease progression at both the virological and immunological levels (12). The HIV-driven V\( \delta \)2 cell depletion/inactivation is consistent with the definition of viral immune evasion

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Address correspondence to Lucia Conti, lucia.conti@iss.it.

* Present address: Marco Cardone, Office of Biotechnology Products, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland, USA; Kyojiro N. Ikeda, Dunn School of Pathology, University of Oxford, Oxford, United Kingdom.

S.G. and L.C. were joint principal investigators.

This paper is dedicated to Fabrizio Poccia, who introduced us to the fascinating world of \( \gamma \delta \) T lymphocytes.

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mechanisms and suggests a crucial involvement of Vβ2 T cells in the early control of infection as well as in the response to opportunistic pathogens (13, 14). Despite this evidence, the causes of their dysfunctions still remain to be clarified. γδ T cells lack the CD4 receptor and are generally considered not susceptible to HIV-1 infection; thus, indirect mechanisms have been proposed for finally explaining their dysfunction (15).

Dendritic cells (DC) are among the first cells targeted by HIV at the mucosal sites and are actively involved in spreading the virus to susceptible CD4+ T lymphocytes (16). Given their pivotal role in marshalling immune responses, these cells have been exploited by the virus to escape antiviral immunity. Several studies have reported a decline in the number of blood DC as well as DC-associated dysfunctions in HIV-infected individuals (17). Moreover, both phenotypic and functional alterations have been described for circulating DC and monocyte-derived DC (MDDC) exposed to infectious HIV-1 or to viral products (18). In particular, it has been shown that exposure of MDDC either to the virus or to its envelope glycoprotein gp120 impairs their maturation induced by Toll-like receptor (TLR) or CD40 triggering (19).

A number of groups, including ours, previously reported that DC play a crucial role in the activation/expansion of γδ T lymphocytes in response to phosophoantigens (20–24) and that, reciprocally, activated lymphocytes deliver maturation stimuli to DC (21, 22, 24, 25). In particular, DC are strictly required for the activation of γδ T cells by aminobiphosphonate antigens such as pamidronate (PAM), whereas they only potentiate the expansion of these cells following their direct activation by phosphomonoester antigens such as isopentenyl pyrophosphate (IPP). Growing evidence indicates that the interplay of γδ T lymphocytes with DC and other immune cell populations can actively contribute to the orchestration of immune response by bridging innate to adaptive immunity (26, 27). However, the role of DC/γδ T cell cross talk in HIV infection has been poorly investigated.

We report here that the interaction of γδ T lymphocytes with HIV-1-exposed DC results in a significant reduction of lymphocyte expansion and gamma interferon (IFN-γ) production in response to antigens. The impairment of γδ T cell proliferation directly correlates with HIV-induced suppression of interleukin 12 (IL-12) secretion in DC/γδ T cell cocultures. We also show that, reciprocally, antigen-stimulated γδ T cells exert a control on HIV-1 replication in interacting DC as well as on virus spreading to target CD4+ T lymphocytes.

**MATERIALS AND METHODS**

**Ethics statements.** Healthy donor buffy coats were obtained from Centro Trasfusionale, Sapienza University of Rome. Buffy coats were not obtained specifically for this study. Informed consent was not requested because data were analyzed anonymously. Data from healthy donors have been treated by Centro Trasfusionale according to the Italian law on personal data management, “Codice in materia di protezione dei dati personali” (Testo unico D.L. June 30, 2003, no. 196).

**Generation of DC and isolation of γδ T lymphocytes.** Monocytes were isolated from the peripheral blood of healthy donors. Peripheral blood mononuclear cells (PBMC) were separated by Ficoll-Paque density centrifugation and subject to CD14+ selection by immunomagnetic beads (Miltenyi Biotech). This procedure yields a ≥95% pure monocyte population as assessed by analysis of lineage specific markers.

Monocyte-derived DC (MDDC) were generated by culturing monocytes for 5 days in 24-well plates (10⁶/ml) in RPMI medium plus 10% heat-inactivated fetal bovine serum (FBS) in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 (50 ng/ml and 500 U/ml, respectively; Schering-Plough), as previously described (21).

γδ T lymphocytes were isolated from cryopreserved autologous PBMC by positive selection through immunomagnetic beads (Miltenyi Biotech). Purified cells were cultured overnight at 37°C to allow bead release, then extensively washed, and used for coculture experiments.

**HIV-1 infection of DC and DC/γδ T cell cocultures.** HIV-1_bal and HIV-1_int stocks were purchased from Advanced Biotechnologies (Columbia, MD) and consisted of pelleted virions obtained after propagation in primary human macrophages and H9 cells, respectively. The multiplicity of infection (MOI) was determined by the manufacturer in primary macrophage cultures or H9 cells.

Five-day-cultured MDDC were left untreated or exposed to HIV-1 virions (MOI, 0.2) for 24 h, washed, and then left in culture for additional 24 h. Purified autologous γδ T lymphocytes were added (1:1 ratio) and cocultured with DC in the presence or absence of pamidronate (PAM, 10 μg/ml; kindly provided by Novartis Pharma, Origgio, Italy) or isopentenyl pyrophosphate (IPP, 2 μg/ml; Sigma-Aldrich) for 48 h or more, as previously described (21). Cocultures were then phenotypically and functionally characterized. In some experiments, DC were infected with HIV soon after coculture setting or cocultured with γδ T cells in the presence of neutralizing anti-CCL4 monoclonal antibody (MAb) or control MAb (5 μg/ml; R&D Systems).

γδ T cells were also directly exposed to HIV-1_bal (MOI, 0.2) h or 48 h before stimulation with IPP and then analyzed for the expression of activation markers and cytokine production.

**Phenotypic analysis of DC and γδ T cells.** γδ T cell cultures and DC/γδ T cell cocultures were analyzed at 48 h for the expression of cell specific markers by staining with phycoerythrin (PE)-conjugated MAbs to CD25, CD69, HLA-DR, CD86, or MR or isotype-matched control MAb (BD Biosciences). Briefly, 10⁶ cells were preincubated with phosphate-buffered saline (PBS) containing 10% human AB serum to block unspecific Ig binding, then stained with the specific MAbs for 30 min on ice, washed, and analyzed by flow cytometry. At least 10,000 events/sample were acquired by a FACScan cytometer (BD Biosciences). Data analysis was performed by gating on the lymphocyte or DC population and excluding dead cells and debris.

**FITC-dextran uptake.** DC/γδ T cell cocultures were harvested at 48 h and incubated with fluorescein isothiocyanate (FITC)-labeled dextran (0.05 mg/ml; molecular weight [MW], 70,000; Molecular Probes) for 40 min at 37°C. Cells were then extensively washed and analyzed by flow cytometry by gating on the DC population, as previously described (28).

**Lymphocyte proliferation assays.** Purified γδ T cells were stained with 5 μM carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes) for 4 min at room temperature (RT) as previously described (29). Labeled cells were then washed, suspended in complete medium (10⁶ cells/ml), and cocultured in 96-well plates with autologous uninfected or HIV-infected DC (1:1 ratio) in the presence or absence of PAM or IPP. After 6 days, cell proliferation was analyzed by flow cytometry by gating on the lymphocyte population. Alternatively, γδ T cell proliferation was assessed by [³H]thymidine incorporation. Briefly, DC exposed or not to HIV-1 for 48 h were cocultured with autologous γδ T cells (1:3 ratio) for 5 days in RPMI medium plus 5% AB serum in the presence or absence of PAM. During the final 16 h of culture, cells were pulsed with 0.5 μCi of [³H]thymidine (specific activity, 5 Ci/mmol; Amersham Biosciences, United Kingdom) and counted as described previously (21).

**Cytokine and chemokine release determination.** Culture medium collected from 48-h or 6-day DC/γδ T cell cocultures, or from 6-day γδ T cell cultures, was analyzed for IFN-γ (Biolegend), IL-17, tumor necrosis factor alpha (TNF-α), IL-12, IL-10, CCL4 (R&D Systems), and IL-23 (Bender MedSystems) production by enzyme-linked immunosorbent assay (ELISA). The release of IL-10 and CCL4 was also measured in supernatants from DC exposed or not to HIV-1 for 48 h.
Determination of HIV replication and virus transfer to CD4+ T lymphocytes. DC exposed to HIV-1 for 48 h were left untreated or cocultured with autologous γδ T cells for 5 to 6 days, and the extent of virus replication was then analyzed by intracellular p24\textsubscript{ gag } expression. Cells were fixed with 4% paraformaldehyde (PFA), then permeated with 70% ethanol, and stained with PE-conjugated HIV-1 p24\textsubscript{ gag } MAb (KC57 Coulter Clone) for 30 min at 4°C as previously described (30). Labeled cells (20,000 events/sample) were acquired by a FACScan cytometer (BD Biosciences). Data analysis was performed by gating on the DC population and by excluding dead cells and debris. Previous control experiments in which DC were exposed to the virus in the presence of zidovudine (AZT) had confirmed that the fraction of infected cells detected is not biased by cell membrane-associated p24\textsubscript{ gag }.

For virus transfer experiments, HIV-infected DC, exposed to γδ T cells as described above, were then cocultured (1:5 ratio) for 3 to 6 days with purified CD4\textsuperscript{+} T cells, previously activated for 3 days with phytohemagglutinin (PHA, 2 μg/ml; Sigma), in the presence of IL-2 (50 U/ml; Roche). The extent of virus replication in T lymphocytes, after removal of γδ T cells from the cocultures by positive selection, was monitored by staining with PE-conjugated HIV-1 p24\textsubscript{ gag } MAb and analyzing positive cells after gating on the lymphocyte population. A total of 20,000 events/sample were acquired.

Chemoattract assay. γδ T cell migration was performed in 24-well Transwell culture chambers (Costar, NY). Briefly, 5 × 10\textsuperscript{5} resting cells were washed, suspended in serum-free medium, and loaded in the upper compartment (2 wells/condition). RPMI medium plus 10% FBS or conditioned medium (CM) from uninfected or HIV-infected DC were added to the lower compartment. After 3 h of incubation at 37°C, cells had migrated into the lower compartment through the 3-μm-pore-size polycarbonate filters were collected and counted. Total γδ T cells were also stained, before and after migration, with FITC-conjugated anti V\textsubscript{δ1} TCR MAb or isotype-matched control MAb (Miltenyi Biotech) for 20 min at 4°C, then washed, and analyzed by flow cytometry. In some experiments, γδ T cells were either exposed to HIV-DC-derived CM that had been previously incubated with neutralizing anti-CCL4 MAb or control MAb (5 μg/ml; R&D Systems; 40 min at 37°C) or treated with the CCR5 inhibitor TAK779 (5 μM; kindly provided by the National Institutes of Health AIDS Research and Reference Reagent Program) for 40 min at 37°C before exposure to HIV-DC CM.

Statistical analysis. Paired Student’s t test and Pearson’s correlation were used to analyze data. A P value of <0.05 was considered statistically significant.

RESULTS

HIV-1-exposed DC exhibit a reduced capacity to promote γδ T cell proliferation and IFN-γ production in response to pho-shoantigens. To test the hypothesis that HIV-induced dysfunctions in DC affect their activating interaction with γδ T lymphocytes, purified γδ T cells were stimulated with PAM in the presence of HIV-1\textsubscript{lat}-infected autologous DC and then analyzed for their capacity to produce IFN-γ. A significant reduction in IFN-γ secretion was found when γδ T cells were cocultured with HIV-exposed DC (HIV-DC) compared to control DC (Fig. 1A, all donors). However, within the group of 22 donors analyzed, two different subgroups were identified, referred to as sensitive and resistant donors (Fig. 1A). Specifically, HIV-DC/γδ T cell cocultures from sensitive donors (12 out of 22 [55%]) showed a significant reduction of IFN-γ production (fold change [FC] > 2), whereas no change occurred in cocultures from resistant donors. In contrast, comparable levels of TNF-α expression were found in cocultures of γδ T cells with either HIV-exposed or unexposed control DC from both donor subgroups (mean ± standard deviation [SD] of 1,986 ± 546.30 pg/ml versus 1,735 ± 630 pg/ml; n = 14).

To investigate whether the interaction of γδ T cells with HIV-DC affects their proliferation rate, CFSE-labeled, PAM-stimulated γδ T cells were cocultured with autologous HIV-DC or control cells for 5 to 6 days in the absence of IL-2, as previously described (29), and lymphocyte expansion was monitored by flow cytometry. As shown in Fig. 1B, a lower proportion of proliferating lymphocytes was found in the cocultures with HIV-DC when all the donors were analyzed. Again, a significant reduction of PAM-induced γδ T cell proliferation (FC > 2) was observed only in the 12 sensitive donors described above (Fig. 1B and C). Consistently with the reduced expansion, γδ T cells exposed to HIV-DC from sensitive donors produced significantly smaller amounts of IFN-γ in response to PAM (Fig. 1D). The expression of IL-17 was also monitored, but this cytokine was not found under any experimental condition (data not shown). The inhibitory effect of HIV-1\textsubscript{lat}-exposed DC on PAM-induced γδ T cell expansion was also observed, in sensitive donors, when proliferation was assessed by [3H]thymidine incorporation and was reproduced when DC were exposed to HIV-1\textsubscript{lat} (Fig. 1E), thus indicating that the impairment of γδ T lymphocyte activity occurs with both R5 and X4 viral strains.

We then investigated whether the reduced expansion of γδ T cells cocultured with virus-exposed DC was associated with alterations of lymphocyte activation marker expression following TCR stimulation. We found that despite the reduction of γδ T cell proliferation and IFN-γ production observed for sensitive donors, the upregulation of CD25 and CD69 on PAM-stimulated lymphocytes from these donors was not significantly affected by HIV-DC coculture (Fig. 1F). In contrast, the induction of major histocompatibility complex (MHC) class II and of the costimulatory molecule CD86 was significantly enhanced in lymphocytes from infected cocultures with respect to control cultures (Fig. 1F).

It has been previously shown that DC promote γδ T cell expansion induced by the phosphomonoester antigen IPP in the absence of IL-2, although they are not required for lymphocyte activation by this antigen (22). Based on this evidence, the effect of HIV-DC on IPP-induced γδ T cell expansion and IFN-γ production was also investigated. As reported in Table 1, virus-exposed DC from sensitive donors promote markedly reduced IPP-induced proliferation and IFN-γ secretion compared to those of control DC, suggesting that the negative effect exerted by HIV-DC is an intrinsic capacity of the latter and does not depend on the antigen used. Although the total γδ T cell population used also contains a proportion of V\textsubscript{δ1}\textsuperscript{+} TCR-expressing cells (ranging from 28 to 33% under our conditions), we believe that the negative effect of HIV-DC is exerted on V\textsubscript{δ2} TCR-positive cells, which are selectively enriched upon stimulation with phosphoantigens.

Resting γδ T lymphocytes express functional CCR5 and CXCR4. HIV-1 coreceptors that could transduce virus-induced signals in the absence of CD4. We thus analyzed whether direct exposure of γδ T cells to HIV-1 could affect their activation following TCR stimulation, independently of DC. The results of these experiments revealed that the exposure of γδ T lymphocytes to HIV-1\textsubscript{lat} simultaneously or 48 h before IPP stimulation, does not affect their capacity to proliferate and to produce IFN-γ (data not shown).

HIV-1-exposed DC from sensitive donors undergo reduced functional maturation and fail to produce IL-12 in response to antigen-stimulated γδ T lymphocytes. We previously demonstrated that once activated, γδ T cells reciprocally induce the full maturation of cocultured DC (21) and that HIV- or gp120-ex-
posed DC, although exhibiting an activated phenotype, fail to undergo functional maturation upon TLR or CD40 triggering (19).

We thus analyzed the activation state of HIV-DC after coculture with PAM-stimulated /H9253/H9254 T lymphocytes by measuring their capacities to produce IL-12, to endocytose dextran, and to upregulate specific surface molecules. As shown in Fig. 2A (all donors), HIV-DC exhibited a different IL-12 secretion profile, with significantly lower levels of cytokine being released, with respect to uninfected control cells following interaction with PAM-stimulated lymphocytes. Interestingly, by subgrouping the donors on the basis of /H9253/H9254 T cell response, statistically significant virus-induced IL-12 suppression was observed only in the sensitive donors (Fig. 2A). Conversely, comparable amounts of IL-10 were detected in DC/lymphocyte cocultures from the latter donors, independently
of virus exposure (Fig. 2B). The expression of IL-23 was also found to be induced, at very low levels, in both infected and uninfected cocultures (data not shown).

For sensitive donors, the induction of phenotypic marker expression was also analyzed. As shown in Fig. 2C (upper graph, iDC), unstimulated HIV-DC exhibited comparable levels of HLA-DR and higher levels of CD86 with respect to uninfected cells. Upon coculture with PAM-stimulated γδ T lymphocytes, the expression of CD86 was upregulated at comparable levels in HIV exposed and unexposed DC (Fig. 2C, lower graph, mDC). Conversely, a higher proportion of mannose receptor (MR)-expressing cells, indicative of a lower degree of maturation, was maintained within virus-exposed DC cultures with respect to uninfected control DC following coculture with γδ T lymphocytes (Fig. 2C). As a consequence of increased MR expression, γδ T

### TABLE 1

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<thead>
<tr>
<th>Culture condition</th>
<th>% proliferating cells</th>
<th>IFN-γ (ng/ml)</th>
</tr>
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<tbody>
<tr>
<td>DC/γδ IPP</td>
<td>36.25 ± 4.99</td>
<td>14.06 ± 3.21</td>
</tr>
<tr>
<td>HIV-DC/γδ IPP</td>
<td>13.75 ± 4.78*</td>
<td>4.83 ± 1.26*</td>
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* CFSE-labeled γδ T lymphocytes were cocultured, in the presence of IPP (2 μg/ml), with HIV-1-infected or uninfected DC (1:1 ratio) for 6 days and then analyzed for the extent of cell proliferation and for IFN-γ release in the culture medium. At the time of analysis, cell counts were 1.12 ± 0.19 × 10^6 in control cocultures and 4.8 ± 1.1 × 10^5 in infected ones (n = 4). No substantial differences in cell viability were observed in cocultures with infected DC with respect to control cocultures. Flow cytometry analysis was performed by gating on the lymphocyte population. Mean values ± SDs from 4 (proliferating cells) and 5 (IFN-γ) independent responsive donors are shown. *, P < 0.05.

**FIG 2** Phenotypic and functional maturation of HIV-DC following interaction with activated γδ T cells. (A) Unexposed (control) and HIV-exposed (HIV) DC from 22 blood donors were cocultured for 48 h with γδ T lymphocytes in the presence of PAM, and IL-12 release in the culture supernatants was measured by ELISA. Results from all (n = 22), sensitive (n = 12), and resistant (n = 10) donors are shown (mean ± SD with range is presented for each group). (B) IL-12 and IL-10 levels (mean values ± SDs) released in HIV exposed or control DC/γδ T cell cocultures from 12 sensitive donors are reported. (C) HIV-exposed and control DC were analyzed for the expression of the indicated surface markers, as immature cells (iDC) or after maturation induced by 48 h of coculture with γδ T lymphocytes (mDC). Mean values ± SDs from five independent sensitive donors are shown. (D and E) γδ T cell-cocultured DC were incubated with FITC-dextran for 40 min at 37°C and then analyzed by flow cytometry. A representative experiment from one sensitive donor out of 5 is shown in panel D. Open histograms represent the background staining of cells incubated with FITC-dextran at 0°C; numbers indicate the percentages of positive cells. Mean values ± SDs from five independent sensitive donors are shown in panel E. Data analysis was performed by gating on the DC population and excluding dead cells and debris. *, P < 0.05; ***, P < 0.001 (comparison between control and HIV).
Reduced modifications in DC observed in sensitive donors affect T cell properties, DC were cocultured with PAM-stimulated /H9253 IFN-endoxylose dextran-FITC (Fig. 2D and E).

**Fig. 3** Restoration of lymphocyte proliferation and IFN-γ production by exogenous IL-12. CFSE-labeled γδ T lymphocytes were seeded with DC (1:1 ratio) either at the time of HIV-1 infection (DC/γδ + HIV) or after 48 h of DC exposure to the virus (HIV-DC/γδ), in the presence or absence of rIL-12 (1 ng/ml, Peprotech). The extent of cell proliferation (A and B) and the production of IFN-γ (C) were monitored after 6 days of coculture by flow cytometry and ELISA, respectively. A representative experiment from 1 out of 5 sensitive donors is reported in panel A. The percentage of proliferating cells is indicated in panel A. For panels B and C, all the cocultures were activated with PAM. Mean values ± SDs from 5 independent donors are reported. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Exogenous IL-12 restores γδ T lymphocyte proliferation and IFN-γ production. In order to elucidate whether the virus-induced modifications in DC observed in sensitive donors affect γδ T cell properties, DC were cocultured with PAM-stimulated γδ T lymphocytes either 48 h postinfection, as described above, or concomitantly with infection, and the effects on lymphocyte proliferation and IFN-γ production were then examined. As shown in Fig. 3A and B, the negative effect of HIV-DC on γδ T cell proliferation was observed only when the latter were cocultured with DC preexposed to the virus, whereas the simultaneous exposure of DC to HIV and lymphocytes did not significantly affect proliferation. The simultaneous treatment also led to normal IL-12 secretion (data not shown). Interestingly, when recombinant IL-12 (rIL-12) was added to the cocultures of γδ T cells and DC preexposed to HIV, the impairment of lymphocyte proliferation in response to PAM was almost completely prevented (Fig. 3A and B). Similarly, reduced levels of IFN-γ were detected only in virus-preexposed cocultures, and the production of IFN-γ was rescued by IL-12 (Fig. 3C). Consistent with these results, a direct correlation was found between IL-12 expression in HIV-DC and IFN-γ production in interacting γδ T lymphocytes (n = 22; r² = 0.86; P < 0.0001). These results indicate that in sensitive donors, virus-induced changes in DC are responsible for the impairment of γδ T cell activity, and the results also point to HIV-induced loss of IL-12 production as a major cause.

γδ T lymphocytes are recruited by HIV-1-infected DC and control virus replication and spreading. To dissect the relevance of the dysfunctional DC/innate lymphocyte cross talk in HIV pathogenesis, we evaluated whether γδ T cells could be recruited in close proximity to virus-infected DC. To this end, purified resting γδ T cells, isolated from the peripheral blood of healthy donors, were analyzed for their capacity to migrate toward conditioned medium (CM) of DC previously infected with HIV-1_R4, in Transwell-based chemotaxis assays. As shown in Fig. 4A, a basal rate of migration was observed when lymphocytes were exposed to CM of control DC with respect to medium alone. However, γδ T cells exhibited a significantly higher capacity to migrate when stimulated by CM from HIV-DC (Fig. 4A). Interestingly, Vδ2 TCR-expressing cells were found to be the prevalent subset recruited (Fig. 4B). Moreover, as we observed an induction of CCL4 upon exposure of DC to HIV (Fig. 4C), the role of CCR5 and CCR5-binding chemokines in driving γδ T cell migration toward HIV-DC was explored. As shown in Fig. 4D, both neutralization of CCL4 in HIV-DC CM and γδ T cell exposure to the CCR5 inhibitor TAK779 significantly reduced the proportion of migrating cells. These results were obtained irrespective of the donor type analyzed and indicate that the interaction between DC and γδ T lymphocytes can be enhanced when DC are exposed to the virus.

This observation, together with the rapid depletion of γδ T cells described for acute HIV-1 infection, suggest a functional role of this cell subset in the early control of virus replication. We thus analyzed whether the interaction between HIV-infected DC and γδ T lymphocytes could affect the replication efficiency of the virus and its spreading within the DC cultures. To test this hypothesis, DC infected with HIV-1_R4 for 48 h were left untreated or
allowed to interact with autologous γδ T lymphocytes in the presence of PAM as described above, and after 6 to 7 days of coculture, the intracellular expression of p24 was assessed by flow cytometry. As shown in Fig. 5A and B, a low but detectable proportion of virus-exposed DC expressed the viral antigen 7 days postinfection. Interestingly, a significant decrease in the number of infected cells was observed following interaction of DC cultures with PAM-activated γδ T lymphocytes (Fig. 5A and B). Notably, stimulation of HIV-DC with PAM in the absence of γδ T cells did not decrease the percentage of infected DC, suggesting that PAM-activated lymphocytes and not the stimulus per se were responsible for the control of virus replication (data not shown). HIV exposure of DC results not only in productive infection of these cells but also in the transfer of the virus to susceptible CD4+ T lymphocytes (16). To further investigate whether the suppressive effect of activated γδ T cells on HIV spreading among DC could also regulate their efficiency of virus transfer to CD4+ T lymphocytes, HIV-DC, exposed or not to PAM-stimulated γδ T cells, were cocultured with preactivated CD4+ αβ T lymphocytes and the expression of p24 within the lymphocyte population was monitored. As shown in Fig. 5C and D, CD4+ T cells were infected upon interaction with HIV-DC. In contrast, a significantly lower number of p24+ T lymphocytes was detected when infected DC had previously interacted with PAM-stimulated γδ T cells (Fig. 5C and D). The suppressive effect on HIV-1 replication in DC was also observed when γδ T lymphocytes were activated with IPP and then added to the infected DC cultures (data not shown). In contrast to what was observed for the HIV-DC-mediated impairment of γδ T cell functions, the suppression of HIV replication/spreading was a more general phenomenon, independent of IL-12 production in DC, as it occurred in both sensitive and resistant donors. Notably, comparable levels of CCL4 were detected in PAM-activated cocultures independently of HIV exposure (22.24 ± 4.18 ng/ml in control versus 23.08 ± 5.0 ng/ml in infected cocultures; n = 5), and neutralization of this chemokine during HIV-DC/γδ T cell cross talk significantly increased both the proportion of p24-positive DC and that of cocultured CD4+ T lymphocytes (Fig. 5E and F). In support to this finding, we observed that PAM-activated γδ T cells can suppress HIV replication in CD4+ T lymphocytes when the latter are infected with free R5 HIV-1 na but not X4 HIV-1 n (data not shown).

**DISCUSSION**

The cross talk between DC and γδ T lymphocytes represents a major control mechanism for an integrated immune response to infections and may be exploited as a target for pathogen-induced...
immune escape (26). Notably, the number and functions of both cell populations are dramatically affected in HIV-1 infection (14). In this work, we report that exposure of DC to HIV-1 induces functional alterations in these cells that, in turn, negatively influence their cross talk with \( \gamma\delta \) T lymphocytes. We provide the first demonstration that HIV-exposed DC deliver negative signals to aminobiphosphonate-stimulated \( \gamma\delta \) T lymphocytes, resulting in impaired cell proliferation and type 1 cytokine production. Although independent of the virus strain, this effect was observed in the 55% of donors analyzed (sensitive donors). In this regard, donor-to-donor variability in the response to HIV-1 infection has been previously reported for MDDC. In particular, Shan and co-workers demonstrated that IL-10 production, induced by gp120 derived from selected HIV-1 strains, occurred in MDDC from only 62% of donors (31). However, we have been unable to demonstrate any involvement of IL-10 in discriminating between sensitive and resistant donors, as this cytokine is not induced by the virus in DC from sensitive or resistant donors (data not shown). Furthermore, the levels of IL-10 released in the cocultures with activated lymphocytes are not affected by the virus (Fig. 2B). In contrast with other studies correlating the different capacity of HIV-infected DC to produce inflammatory cytokines with gender, we failed to find any relationship between the different behavior of the donors analyzed in this study and the sex of the donors (32), as resistant individuals were equally found among women and men (data not shown). Thus, it remains to be determined.
whether complex host genetic and/or epigenetic factors could be responsible for this donor-related variability. However, it should be taken into consideration that variations in the early response to the virus may exist and that these early events could be responsible for the different outcomes of the disease in different donors.

Interestingly, we demonstrate that the donor-dependent variation observed is strictly linked to the virus-mediated suppression of IL-12 expression in DC, since the reduced IL-12 secretion in sensitive donors is a major cause of γδ T lymphocyte impairment. HIV-1 infection induces a broad range of host responses, some of which interfere with the regulatory pathways of IL-12 gene expression (33–35). IL-12 production was shown to be impaired in the course of infection, and the relevance of its deficiency in virus-induced pathogenesis is suggested by the finding that exogenous administration of this cytokine restores immune responsiveness of peripheral leukocytes from AIDS patients (36). Furthermore, monocyte-derived DC generated from HIV-infected individuals exhibit an unbalanced IL-12/IL-10 ratio and have a reduced capacity to stimulate IFN-γ secretion in both T lymphocytes (37) and natural killer cells (38). In this regard, it has been reported that IL-12, in addition to exerting a proliferative and Th1-polarizing effect on conventional γδ T lymphocytes, is also critical for the activation and proliferation of γδ T cells (39–43). Here we report that for a proportion of donors, HIV-1 can suppress IL-12 production in DC induced not only by TLR or CD40 stimulation but also following their functional interaction with other immune cells. We also demonstrate that the reduced DC-mediated γδ T cell proliferation and IFN-γ production strongly depend on virus-induced impairment of IL-12 production and that exogenous addition of this cytokine restores lymphocyte functions. Our data are discordant with previously published results showing that exogenous cytokines, including IL-12, could not reverse the functional defects of γδ T cells observed in HIV-infected individuals (40, 44). In those studies, total PBMC isolated from HIV-infected donors, and thus exposed in vivo to the virus, were used for ex vivo stimulation with mycobacterial antigens (40). We report here that normal γδ T lymphocytes from immunocompetent donors can lose their capacity to expand and produce Th1 cytokines in response to specific antigens when allowed to interact with DC functionally impaired by HIV, and we demonstrate that this loss can be prevented in the presence of exogenous IL-12. Although not immediately reflecting the actual situation of infected individuals and with the intrinsic limitations of in vitro studies, our approach aimed to mimic the early phases of γδ T cell damage through the interaction of these T cells with DC, which are among the first cells targeted by the virus during acute infection. Our results showing the capacity of infected DC to produce CCL4 and to attract CCR5-expressing resting γδ T lymphocytes would support this view.

In addition to the reduced IL-12 production, the increased IL-10/IL-12 ratio we observed in infected cocultures might negatively affect γδ T cell function. In this regard, it has been reported that IL-10 inhibits γδ T cell proliferation and IFN-γ production in the presence of monocytes (45). Furthermore, we have recently reported that manipulating the IL-10/IL-12 balance by either blocking endogenous IL-12 or exogenously adding IL-10 significantly decreases IFN-γ production in antigen-stimulated DC/γδ T cell cocultures (46), thus suggesting a crucial role for antigen-presenting cell (APC)-specific cytokines in shaping the γδ T lymphocyte response. In keeping with our findings, Wesch and colleagues reported that the failure of Vδ2 cells from HIV+ individuals to proliferate in response to mycobacterial antigens is not an intrinsic defect of this cell subset and that the responsiveness can be restored following reconstitution with APCs from allogeneic donors (9).

Our results and data from the literature led us to hypothesize that in addition to APC-mediated dysfunctions, direct virus-induced alterations or other still-unexplored mechanisms can cooperate to produce the loss of γδ T cell-mediated immunity observed in HIV+ individuals. In this regard, it has been reported that direct interaction of IPP-expanded γδ T lymphocytes with R5 HIV-1 envelope results in CCR5-α4β7-mediated cell death, and this mechanism has been proposed to contribute to the depletion of this cell population in AIDS patients (14, 47). In the present work, we report that the direct stimulation of freshly isolated γδ T cells with infectious R5 HIV-1 virions before TCR engagement does not affect their capacity to become fully activated and to expand in response to antigens. Although we did not specifically analyze programmed cell death pathways in HIV-1-exposed lymphocyte cultures, we never observed significant virus-induced effects on cell viability (data not shown). However, the different concentration of the envelope glycoproteins as well as the different lymphocyte activation status may explain this apparent discrepancy.

Concerning the putative protective role of γδ T cell-mediated immune response in HIV-1 infection, we study also allowed us to underscore a suppressive effect of activated γδ T cells on virus spreading within DC and to susceptible CD4+ T lymphocytes. Previous papers have reported on the capacity of innate lymphocyte-conditioned medium to inhibit HIV-1 replication in PBMC, macrophages, lymphoid cell lines, and natural killer T cells (48–51). In this study, the inhibitory effect of activated γδ T cells was extended to the DC, which represent one of the first targets and reservoirs for the virus. We also provided the first demonstration that activated γδ T cells can control not only HIV-1 replication within interacting DC but also virus dissemination to CD4+ T lymphocytes. In keeping with the above-discussed papers, we report that activated DC/γδ T cell cocultures produce CCL4, whose neutralization almost completely rescues virus replication. In contrast to our results, Sacchi and colleagues recently reported that although IPP-stimulated γδ T cells are functionally impaired upon exposure to HIV-infected DC, they do not exert any inhibitory effect on p24 release by the latter (52). The different antigen used to activate γδ T cells as well as the different readout system to assess HIV replication may account for this discrepancy. The use, in our study, of aminobiphosphonate antigens, known to selectively activate and expand Vδ2 TCR-expressing cells, led us to hypothesize that this is the main subset functionally impaired by HIV-infected DC and responsible for the suppression of HIV replication. In support of this view are our findings that Vδ2 cells are the prevalent subset able to migrate toward HIV-DC CM (Fig. 4B) and that unstimulated γδ T cells fail to suppress HIV spreading (data not shown). However, an involvement of Vδ1 TCR-positive cells, which could be activated through their interaction with HIV-infected cells as previously reported (51), cannot be excluded.

Independently of the mechanism and subset involved, the suppression of HIV spreading by activated γδ T cells in vitro points to a possible role for this lymphocyte subset in the control of infection in HIV+ individuals that contributes to explain the reason for its early depletion/inactivation. Although the suppressive effect of activated γδ T cells on HIV replication was observed in all the
donors analyzed, the anti-HIV activity of these cells could be controlled by the extent of their activation. This would lead to speculation that a less effective antiviral response is induced in those individuals in whom a dysfunctional DC/lymphocyte cross talk occurs.

In conclusion, it is reasonable to hypothesize that γδ T cell-activating antigens accumulate during HIV infection, in infected or bystander cells, and could induce the expansion of this lymphocyte subset, thus mediating protective effects. Based on our results and on data from the literature, fully activated γδ T lymphocytes could both directly control HIV replication and exert cytokine and immune regulatory activities toward infected cells. However, HIV-induced direct lymphocyte death as well as dysregulated cytokine production in APCs, or other still-unknown mechanisms, could interfere with this potentially protective mechanism by either deleting these cells or negatively affecting their functions.

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