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Dendritic Cell Precursors Are Permissive to Dengue Virus and Human Immunodeficiency Virus Infection

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CD14$^+$ interstitial cells reside beneath the epidermis of skin and mucosal tissue and may therefore play an important role in viral infections and the shaping of an antiviral immune response. However, in contrast to dendritic cells (DC) or blood monocytes, these antigen-presenting cells (APC) have not been well studied. We have previously described long-lived CD14$^+$ cells generated from CD34$^+$ hematopoietic progenitors, which may represent model cells for interstitial CD14$^+$ APC. Here, we show that these cells carry DC-SIGN and differentiate into immature DC in the presence of granulocyte-macrophage colony-stimulating factor. We have compared the CD14$^+$ cells and the DC derived from these cells with respect to dengue virus and human immunodeficiency virus type 1 (HIV-1) infection. Both cell types are permissive to dengue virus infection, but the CD14$^+$ cells secrete the anti-inflammatory cytokine interleukin 10 and no tumor necrosis factor alpha. Regarding HIV, the CD14$^+$ cells are permissive to HIV-1, release higher p24 levels than the derived DC, and more efficiently activate HIV Pol-specific CD8$^+$ memory T cells. The CD14$^+$ DC precursors infected with either virus retain their DC differentiation potential. The results suggest that interstitial CD14$^+$ APC may contribute to HIV-1 and dengue virus infection and the shaping of an antiviral immune response.

The presence of dendritic cells (DC) within mucosal surfaces, skin, and blood, as well as their ability to take up antigens at these sites, predispose DC to be primary targets for viral infection (24). The findings that C-type lectin viral receptors, such as DC-SIGN (10, 11) or *N*-acetylgalactosamine-specific C-type lectin (41, 44), are expressed by interstitial antigen-presenting cells (APC) beneath the epidermis of skin and mucosal tissue (19, 23, 43, 44) have underscored the role of these cells in viral dissemination and the antiviral immune response (41, 43).

The interstitial myeloid APC population is heterogeneous, adapting to local tissue-specific growth and differentiation factors. By examining CD1a and CD14 expression, one can distinguish two subsets, CD1a$^+$ CD14$^-$ and CD1a$^-$ CD14$^+$ cells. It is thought that the former corresponds to interstitial DC or transient Langerhans cells, and DC that have a closely matched phenotype can be generated using granulocyte-macrophage colony-stimulating factor (GM-CSF) in vitro from monocytes (12, 37) or from CD34 progenitors (2, 3). Concerning the CD14$^+$ subset, it has been suggested that they are DC precursors (26, 32), an idea supported by the finding that a subset of CD14$^+$ cells containing Birbeck granules can differentiate into Langerhans-type DC (25). These CD14$^+$ cells differ from monocytes, likewise myeloid DC precursors, by expression of specific markers, such as coagulation factor XIIIa or the C-type lectin DC-SIGN. By culturing a CD34$^+$ progenitor-derived CD14$^+$ subpopulation in M-CSF (4, 7), cells can be obtained that share with interstitial CD14$^+$ cells not only CD14 but also factor XIIIa, tumor necrosis factor-related activation-induced cytokine (TRANCE) (45), and receptor activator of NF-κB (RANK) (1). The cells can convert directly into mature DC when stimulated with lipopolysaccharide (LPS) and cultured on CD40 ligand-transfected fibroblasts (7).

For simplicity, we shall refer to these M-CSF-cultured CD14$^+$ cells as CD14$^+$ DC precursors (preDC). We found that preDC express DC-SIGN, mannose receptor, and the I-type lectin sialoadhesin and rapidly convert into CD1a$^+$ CD14$^-$ immature DC in the presence of GM-CSF–interleukin 4 (IL-4)–transforming growth factor beta (TGF-β). Because dengue virus (DV) and human immunodeficiency virus (HIV) use DC-SIGN as a coreceptor (10, 31, 42) and because their mode of infection renders preDC and DC likely early cell targets, we compared preDC and the CD14$^+$ precursor-derived DC with respect to DV and HIV infectivity and cell response. We found that preDC are infected by DV, remain undifferentiated, and secrete IL-10. These cells are also infected by macrophage-tropic HIV, produce higher levels of Gag p24 protein, and stimulate more Pol-specific CD8$^+$ T cells than DC. HIV type 1 (HIV-1)- or DV-infected preDC remain capable of converting into immature DC. These results suggest that interstitial CD14$^+$ cells may be targets of primary viral infection, supporting viral replication. As potential DC precursors, the cells may serve as vectors for viral dissemination and may modulate the antiviral immune response.

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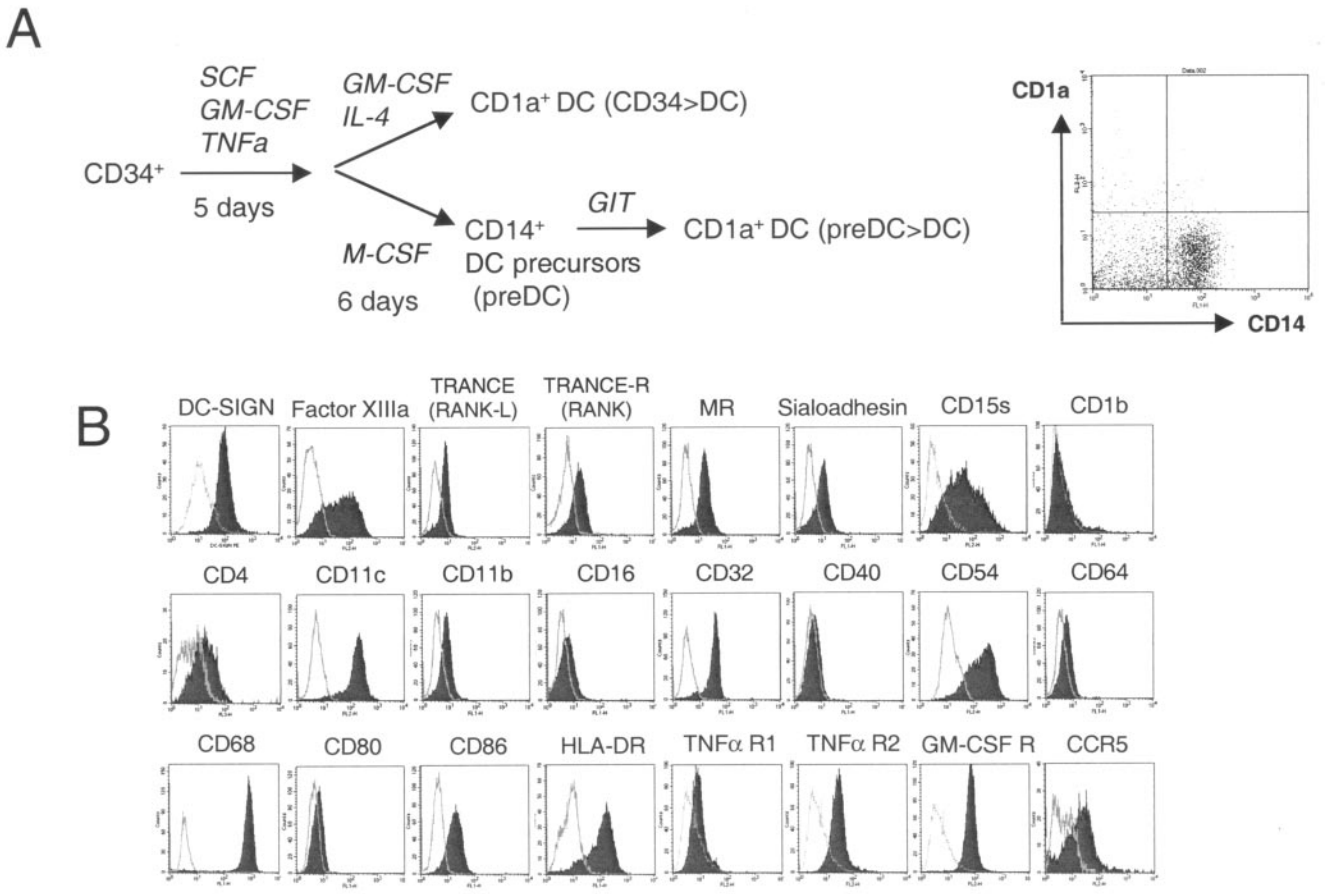

FIG. 1. Generation of $CD14^+$ cells from $CD34^+$ hematopoietic progenitors. (A) Schematic overview of the culture procedures to obtain $CD14^+$ preDC and DC. $CD34^+$ progenitors isolated from umbilical cord blood were cultured in complete medium containing SCF, GM-CSF, and TNF-α (4, 7). After 5 days, the cells were harvested, washed, and cultured for 6 days in M-CSF. $CD14^+$ cells were purified and allowed to differentiate to DC in the presence of GM-CSF–IL-4–TGF-β (*GIT*). Alternatively, DC were obtained by growing the day 5 cell population in GM-CSF–IL-4. Shown is the expression profile of CD1a versus CD14 of the cell population obtained after culture in M-CSF and before cell purification. (B) The antigenic phenotype of $CD14^+$ positively selected cells was determined by flow cytometry using specific antibodies. The data are representative of more than five cord blood samples. All markers (except for intracellular factor XIIIa and CD68) were detected at the cell surface. White histograms represent isotype controls, and specific labeling is shown in gray.

MATERIALS AND METHODS

Cell culture. Cells were cultured in complete RPMI 1640 medium, containing 10% endotoxin-free, 0.8-μm-filtered fetal calf serum (Australian origin; Invitrogen, Cergy-Pontoise, France), 2 mM L-glutamine (Invitrogen), and antibiotics (Invitrogen). Umbilical cord blood was collected after consent and processed according to institutional guidelines. $CD34^+$ progenitors were purified from cord blood using anti-CD34-coated magnetic beads (Miltenyi, Paris, France) and cultured for 5 days in complete medium containing 25 ng/ml stem cell factor (SCF) (R&D Systems, Lille, France), 3 ng/ml tumor necrosis factor alpha (TNF-α) (R&D Systems), and 200 U/ml GM-CSF (Schering-Plough, Kenilworth, NJ), yielding $CD1a^+$ $CD14^-$ and $CD1a^-$ $CD14^+$ precursors. To obtain DC directly from $CD34^+$ progenitors, the day 5 cell population was washed and cultured in 500 U/ml GM-CSF and 5 ng/ml IL-4 (Schering-Plough) for 6 days (Fig. 1A). To obtain $CD14^+$ cells, the day 5 cell population was washed and cultured in 25 ng/ml M-CSF (R&D Systems) for 6 days with an addition of 25 ng/ml M-CSF at day 3. The $CD14^+$ cells were then purified using anti-CD14-coated magnetic beads (Miltenyi). To differentiate the $CD14^+$ DC precursors into interstitial DC, the cells were cultured in 500 U/ml GM-CSF, 5 ng/ml IL-4, and 2 ng/ml TGF-β (R&D Systems) for 72 h (Fig. 1A). Monocyte-derived DC were obtained by culturing monocytes, which were purified using negative cell depletion (Dynal, Compiègne, France) or by counterflow centrifugal elutriation, for 5 days in 500 U/ml GM-CSF and 500 U/ml IL-4 (Peprotech, Rocky Hill, NJ).

Phenotypic analysis. Expression of specific markers was determined by flow cytometry on a FACSCalibur (Beckton-Dickinson [BD], San Jose, CA) using the following monoclonal antibodies (MAb): from BD-Pharmingen (San Diego, CA), CD14-fluorescein isothiocyanate (FITC) (MφP9), CD1a-PE (HI149), CD1b-FITC (M-T101), CD4-CyChrome (RPA-T4), CD32-FITC (FLI8.26), CD40-FITC (5C3), CD54-PE (HA58), CD83-FITC (HB15e), CD86-FITC (FUN-1), and HLA-A2-FITC (BB7.2); from Beckman-Coulter (Roissy, France), HLA DR-FITC (Immu-357), CD80-FITC (MAB104), CD11b-FITC (BEAR1), CD11c-phycoerythrin (PE) (BU15), CD16-FITC (3G8), CD64-FITC (22), GM-CSFRα-PE (SC06), and CD15s (cutaneous lymphocyte antigen; HECA-452); from R&D Systems, CCR5-PE (CTC5) and TRANCE (MAB626); from Caltag Laboratories (Burlingame, CA), TNF-αR1-PE (2H10) and TNF-αR2-PE (4D1B10). Other antibodies recognizing the following antigens were used: CD68-FITC (KP1; Dako, Glostrup, Denmark), FXIIIa (rabbit; Calbiochem, San Diego, CA), and RANK (goat N-20) (Santa-Cruz Biotechnology, Santa Cruz, CA). The anti-DC-SIGN MAb (1B10) was a kind gift from A. Amara (Institut Pasteur, Paris, France), the anti-mannose receptor MAb (DCGM1) receptor was from S. Saeland, Schering-Plough, Dardilly, France, and the antisialoadhesin MAb (HSN-1) was kindly provided by P. Crocker (University of Dundee, Dundee, United Kingdom). Secondary antibodies were from Jackson ImmunoResearch, West Grove, PA, or Caltag Laboratories. Streptavidin-PE was purchased from BD-Pharmingen. The analyses were done using the CellQuestPro software (BD). HLA-A2 donor cord blood was screened using an anti-HLA-A2-FITC-conjugated MAb (BB7.2; BD-Pharmingen).

MLR. $CD14^+$ DC precursors, $CD14^+$ precursor-derived DC, or $CD34^+$ progenitor-derived DC were stimulated for 24 h in culture medium supplemented

with 0.1 μg/ml LPS from *Escherichia coli*, serotype 026:B6 (Sigma-Aldrich, Saint Quentin Fallavier, France). Naive T cells were purified from cord blood $CD34^+$ progenitor-depleted peripheral blood mononuclear cells by positive cell-sorting using anti-CD4-coated magnetic beads (Dynal). After selection, beads were detached using the detach-bead enzyme mix (Dynal). The T-cell population purity was routinely >95%. The mixed lymphocyte reaction (MLR) medium was complete medium supplemented with 5% human AB serum (AbCys, Paris, France) and 1 mM sodium pyruvate (Invitrogen). For the MLR, stimulator cells, washed free of cytokines or LPS, were distributed in triplicate in graded doses in 96-well round-bottom plates (Falcon; BD). Cord blood $CD4^+$ T cells (2×10^4) were added to each well in a final volume of 200 μl. T-cell proliferation was evaluated after 5 days of culture following an overnight incubation with 1 μCi of [^{3}H]methyl-thymidine.

Dengue virus infection and plaque assay. DV type 2, strain 16681, was produced in the *Aedes albopictus* mosquito cell line C6/36. $CD14^+$ DC precursors, $CD14^+$ precursor-derived DC, or $CD34^+$ progenitor-derived DC were infected with live or UV-irradiated DV at different multiplicity of infection (MOI). After 2 h of infection in complete medium containing cytokines, the cells were extensively washed and cultured in complete medium containing cytokines for up to 3 days. At day 2, supernatant virus titers were measured by plaque assay, and supernatant cytokine concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems) according to the manufacturer's instructions. At day 3, cells were harvested for analysis of CD83 expression. In addition, preDC were infected with DV, washed 2 h later, and cultured for 22 h in complete medium containing 10 ng/ml M-CSF (R&D Systems) or placed in complete medium containing 500 U/ml GM-CSF, 5 ng/ml IL-4, and 2 ng/ml TGF-β (R&D Systems). At days 1 and 2, cells were harvested and analyzed for CD1a and CD14 expression.

Cell-free supernatant titers of DV were evaluated by plaque assay as previously described (8). In brief, BHK-21 clone 15 cells were cultured and expanded in alpha-minimal essential medium with 10% fetal calf serum. BHK-21 cells (1.5×10^5 per well in 1 ml) were seeded overnight in 12-well plates at 37°C and 5% CO_2. The next day, the cell supernatant was discarded, and 150 μl per well of serial dilutions of supernatants of DV-infected $CD14^+$ DC precursors or $CD14^+$ precursor-derived DC were added in duplicate and incubated for 1 to 2 h. One and a half milliliters per well of agarose overlay solution (alpha-minimal essential medium, 1% agarose, 5% fetal calf serum) was added to each well and incubated for 5 days. The cells were then fixed in 10% formaldehyde for 1 h at room temperature, and agarose plugs were removed. The plaques were developed in 1% crystal violet–20% ethanol and washed in water.

HIV infection and antigen presentation. Cells were infected with monocytotropic BAL or lymphocytotropic LAI HIV-1 isolates (kindly provided by Gluckman's laboratory, Hôpital Saint Antoine, Paris, France) at a concentration of 50 ng p24 for 10^6 cells. After 3 h of incubation at 37°C with 5% CO_2, the cells were extensively washed and then cultured in complete medium at 10^6 cells per ml. Medium was changed every 3 days, and supernatants were collected and stored at −80°C. HIV replication was assessed using an ELISA kit for p24 detection (Innogenetic, N.V., Belgium). For class I presentation, preDC or $CD34^+$ progenitor-derived DC from HLA-A2 donors were extensively washed 7 days postinfection and then used as antigen-presenting cells in a 16-h gamma interferon (IFN-γ) enzyme-linked immunospot assay, using as effectors cells from an HLA-A2-restricted $Pol_{476-484}$-specific $CD8^+$ T-cell line (28). Two or more effector/APC ratios were systematically tested in triplicate.

RESULTS

Generation of $CD14^+$ cells. In a previous report, we described cells expressing CD14, TRANCE, RANK, and factor XIIIa, generated by culturing $CD34^+$ progenitors in SCF, GM-CSF, and TNF-α followed by incubation of the $CD14^+$ subpopulation in M-CSF (7). Since $CD14^+$ cells could also be obtained from the $CD1a^+$ subpopulation in M-CSF (data not shown), we simplified the protocol by culturing the unseparated cell population in M-CSF. After 6 days, none of the cells expressed CD1a and more than 60% of the population expressed CD14 (Fig. 1A). The majority of the cells were nonadherent, medium-sized, and displayed a round or irregular shape. A minority were adherent to the plastic culture surface with a cell shape ranging from fusiform to round with spread-out cytoplasm. The nonadherent $CD14^+$ population was purified by anti-CD14 MAb-coupled magnetic beads, and Fig. 1B summarizes a representative phenotypic characterization of these cells, which we have called $CD14^+$ preDC. The cells expressed DC-SIGN (11) (CD209), coagulation factor XIIIa (5, 18, 38), TRANCE/RANK-L (45) and TRANCE-R/RANK (1), mannose receptor (9) (CD206), and sialoadhesin (17) (CD169). They also carried CD15s (39) (cutaneous lymphocyte antigen), CD4, CD11c, CD11b, CD32, CD54 (ICAM-1), CD68, CD86, HLA-DR, TNF-αR1, TNF-αR2, GM-CSF-R, and CCR5. The cells expressed little or no CD1b, CD16, CD40, CD64, or CD80. Collectively, the antigenic phenotype renders the in vitro-generated preDC related to the $CD14^+$ interstitial APC subset and different from blood monocytes that lack markers such as factor XIIIa or DC-SIGN.

$CD14^+$ cells are DC precursors. We have previously shown that $CD34^+$ progenitor-derived $CD14^+$ cells can directly convert into mature T-cell-stimulatory DC when treated with LPS and cultured on CD40L-transfected fibroblasts (7). We addressed the question of whether preDC can differentiate into immature DC. As shown in Fig. 2A, a 3-day culture in GM-CSF induced expression of CD1a and loss of CD14. Addition of IL-4 increased the proportion of $CD1a^+$ $CD14^-$ cells to 71%, and addition of TGF-β to GM-CSF–IL-4 further enlarged the proportion of $CD1a^+$ $CD14^-$ cells to 85%. We assessed the differentiation kinetics (Fig. 2B) and observed that the differentiation process in GM-CSF–IL-4–TGFβ was rapid, requiring 48 h to 72 h to reach a maximum percentage of $CD1a^+$ $CD14^-$ cells. The speed with which preDC differentiated into DC varied among donors, since some cells were already fully differentiated into $CD1a^+$ $CD14^-$ DC within 24 h. Routinely, we allowed differentiation to proceed for 3 days, at which time 60 to 90% of the cells were $CD1a^+$ $CD14^-$. The cells did not express Langerin or CCR6 (data not shown), suggesting that they were related to interstitial DC. However, in the presence of GM-CSF–TGF-β, preDC can differentiate into $Langerin^+$ $CCR6^+$ Langerhans-type DC (J.-B. Barbaroux et al., submitted for publication).

To test whether the $CD1a^+$ $CD14^-$ cells were immature DC, we assessed their response to LPS with respect to expression of HLA-DR, CD80, and CD83 and compared the cells with control immature DC, directly derived from $CD34^+$ progenitors in GM-CSF–IL-4 (see Fig. 1A). As shown in Fig. 2C, $CD14^+$ progenitor-derived $CD1a^+$ $CD14^-$ cells responded to LPS by upregulating CD80 and CD83. HLA-DR levels were already relatively high and increased only slightly. This response to LPS was similar to that of the $CD34^+$ progenitor-derived DC. Neither untreated nor LPS-treated preDC were able to prime T cells, even at a high stimulator/responder ratio; however, $CD1a^+$ $CD14^-$ cells derived from preDC and treated with LPS induced vigorous T-cell proliferation (Fig. 2D). The proliferation curve was almost identical to that obtained with control LPS-matured $CD34^+$ progenitor-derived DC. Taken together, the data show that preDC can differentiate rapidly into immature DC in GM-CSF–IL-4–TGF-β, which then mature in response to LPS to become efficient stimulators of naive T cells.

$CD14^+$ DC precursors are permissive to DV but display differences in their cellular response from that of DC. DV binds DC-SIGN (31, 42), replicates rapidly in monocyte-de-

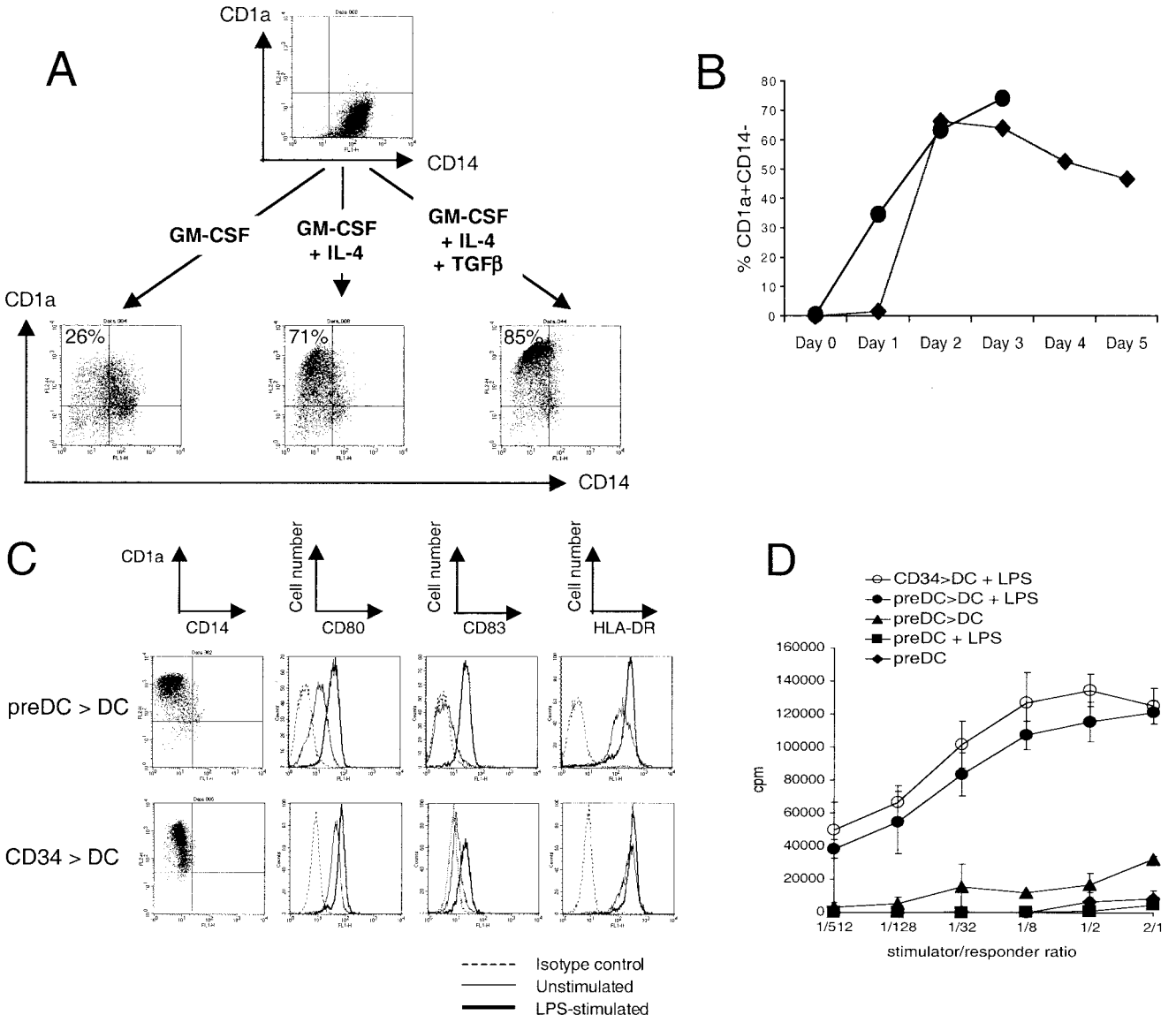

FIG. 2. $CD14^+$ cells differentiate into DC. (A) $CD14^+$ DC precursors were grown for 3 days in either GM-CSF, GM-CSF–IL-4, or GM-CSF–IL-4–TGF-β, and the expression of CD1a versus CD14 was assessed by flow cytometry. The percentage of $CD1a^+$ $CD14^-$ cells is shown. The data are representative of five donors. (B) $CD14^+$ DC precursors were cultured for 3 or 5 days in GM-CSF–IL-4–TGF-β, and the percentage of $CD1a^+$ $CD14^-$ cell was assessed each day. Shown are the results for two different donors. (C) $CD1a^+$ $CD14^-$ cells, obtained either by culturing $CD14^+$ DC precursors in GM-CSF–IL-4–TGF-β (preDC>DC) or directly from $CD34^+$ progenitors in GM-CSF–IL-4 (CD34>DC), were stimulated with LPS, and the expression of CD80, CD83, and HLA-DR was monitored by flow cytometry before and after LPS stimulation. (D) The different APC were cultured in graded doses with allogeneic cord blood-purified $CD4^+$ T cells. T-cell proliferation was measured by [^{3}H]methyl-thymidine incorporation and expressed as mean cpm ± standard deviation for triplicate wells. PreDC were untreated (preDC), stimulated with LPS (preDC+LPS), differentiated into DC without (preDC>DC) or with (preDC>DC+LPS) subsequent LPS stimulation, and compared to control DC derived from $CD34^+$ progenitors and stimulated with LPS (CD34>DC + LPS).

rived DC (20, 27, 31, 42), and triggers TNF-α secretion and DC maturation (20, 27). We compared preDC and the derived DC with respect to DV serotype 2 infection. As shown in Fig. 3A, DV replicated vigorously in preDC-derived DC at 48 h, the peak of the infection kinetics. DV also infected and replicated in preDC, but the infection yielded consistently lower numbers of infectious virions than that of DC. As expected, infection with UV-irradiated DV did not result in production of viral progeny. To check whether the cells exhibited different susceptibilities to apoptosis following DV infection, we analyzed CD14 and CD1a marker expression on preDC and the derived DC, respectively, without electronically excluding dead cells. As shown in Table 1, at 48 h postinfection, the vast majority of the cells were positive for their respective markers. Five days postinfection, about half of the cell population failed to stain for CD14 or CD1a, but there was no marked difference between mock and DV-infected cells. This suggests that DV does not trigger enhanced cell death in either of the two cell types.

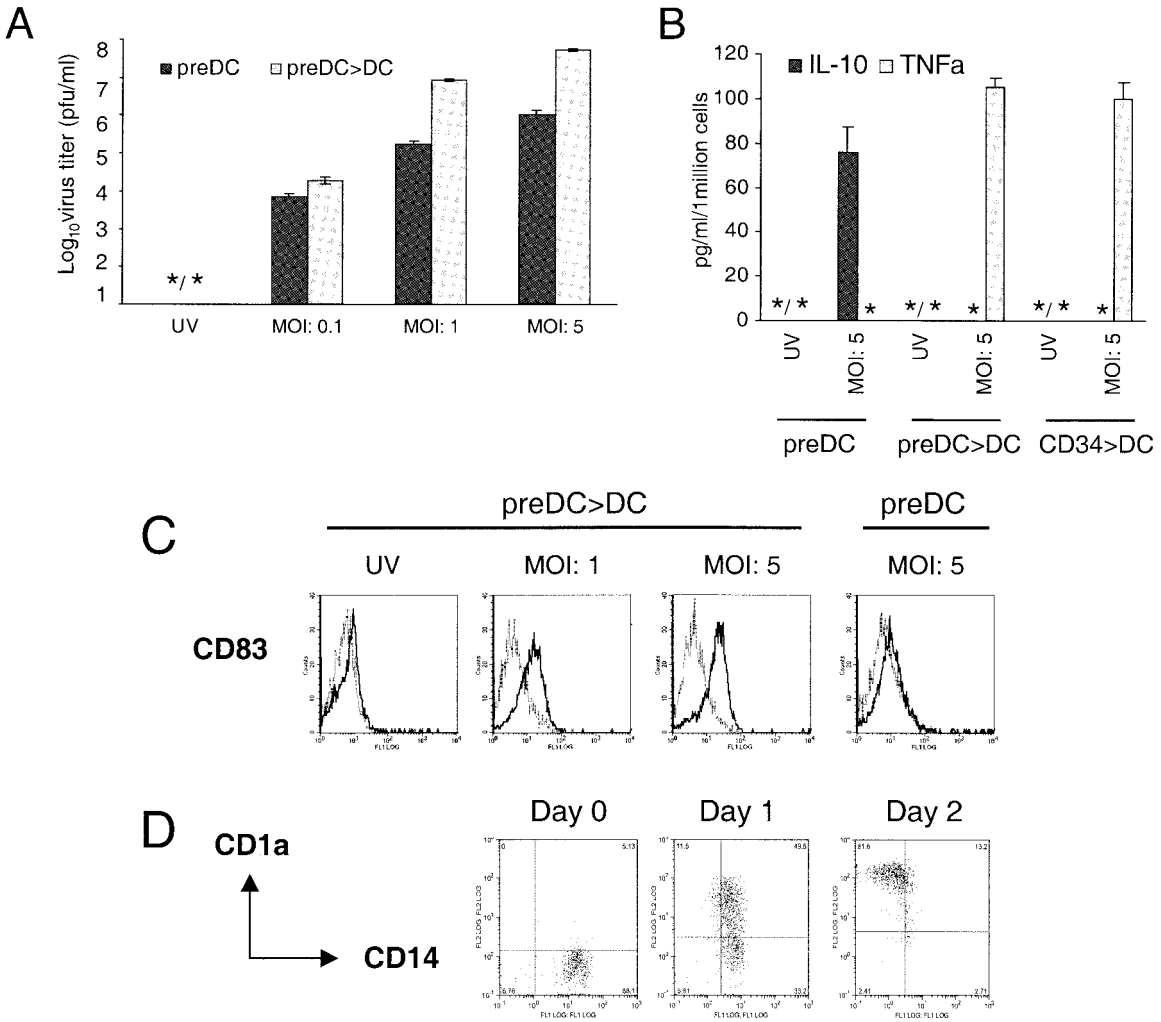

FIG. 3. $CD14^+$ DC precursors are infected by DV type 2. (A) $CD14^+$ DC precursors (preDC) and $CD14^+$ precursor-derived DC (preDC>DC) were incubated with UV-irradiated or live DV at different MOI, as indicated. At 2 days postinfection, the number of virions in the cell supernatant was measured by plaque assay. The data are representative of three different donors. (asterisk, undetected). (B) $CD14^+$ DC precursors (preDC) and $CD14^+$ precursor-derived DC (preDC>DC) or $CD34^+$ progenitor-derived DC (CD34>DC) were infected with UV-irradiated or live DV at MOI 5. Two days after infection, production of IL-10 (black bars) and TNF-α (grey bars) was measured in the supernatants. The data are representative of three different donors. (asterisk, undetected). (C) $CD14^+$ DC precursors (preDC) and $CD14^+$ precursor-derived DC (preDC>DC) were infected with UV-irradiated or live DV at different MOI, as indicated. Three days later, the cell surface expression of CD83 was assessed by flow cytometry. The data are representative of three different donors. (D) Two hours after infection of preDC with DV at an MOI of 1, the cells were cultured in GM-CSF–IL-4–TGF-β. Differentiation into $CD1a^+$ $CD14^-$ immature DC was tested 1 and 2 days afterwards by monitoring CD1a and CD14 expression. The data are representative of three different donors.

We then tested cytokine production and found that DV-infected DC derived from either preDC or $CD34^+$ progenitors produced TNF-α but no IL-10; however, DV-infected preDC released IL-10 but no detectable TNF-α (Fig. 3B). The cytokines were undetectable in supernatants of cells cultured with UV-irradiated DV. We next assessed the effect of DV infection on cell differentiation and observed that infection with replication-competent virus induced CD83 expression by DC as expected (20); however, infected preDC did not express CD83, even at an MOI of 5 (Fig. 3C). DV infection did not trigger preDC to convert to $CD1a^+$ $CD14^-$ DC (data not shown), yet infected preDC differentiated to $CD1a^+$ $CD14^-$ DC with unaltered kinetics when cultured in GM-CSF–IL-4–TGF-β (Fig. 3D). Therefore, both cell types, preDC and DC,

TABLE 1. Percentage of preDC or DC stained positive for CD14 or CD1a, respectively, without excluding dead cells[a]

Cell group	% Positive cells			
	Day 2		Day 5	
	UV	MOI = 5	UV	MOI = 5
preDC	84	87	48	51
preDC>DC	88	90	62	53

[a] PreDC or DC derived from the $CD14^+$ precursors (preDC > DC) were infected with DV at an MOI of 5 (MOI = 5) or with UV-inactivated DV (UV), washed, and cultured. Two days and 5 days afterwards, the percentage of cells positive for CD14 (preDC) or CD1a (DC) was measured, without electronically excluding dead cells on foreward scatter/side scatter channels.

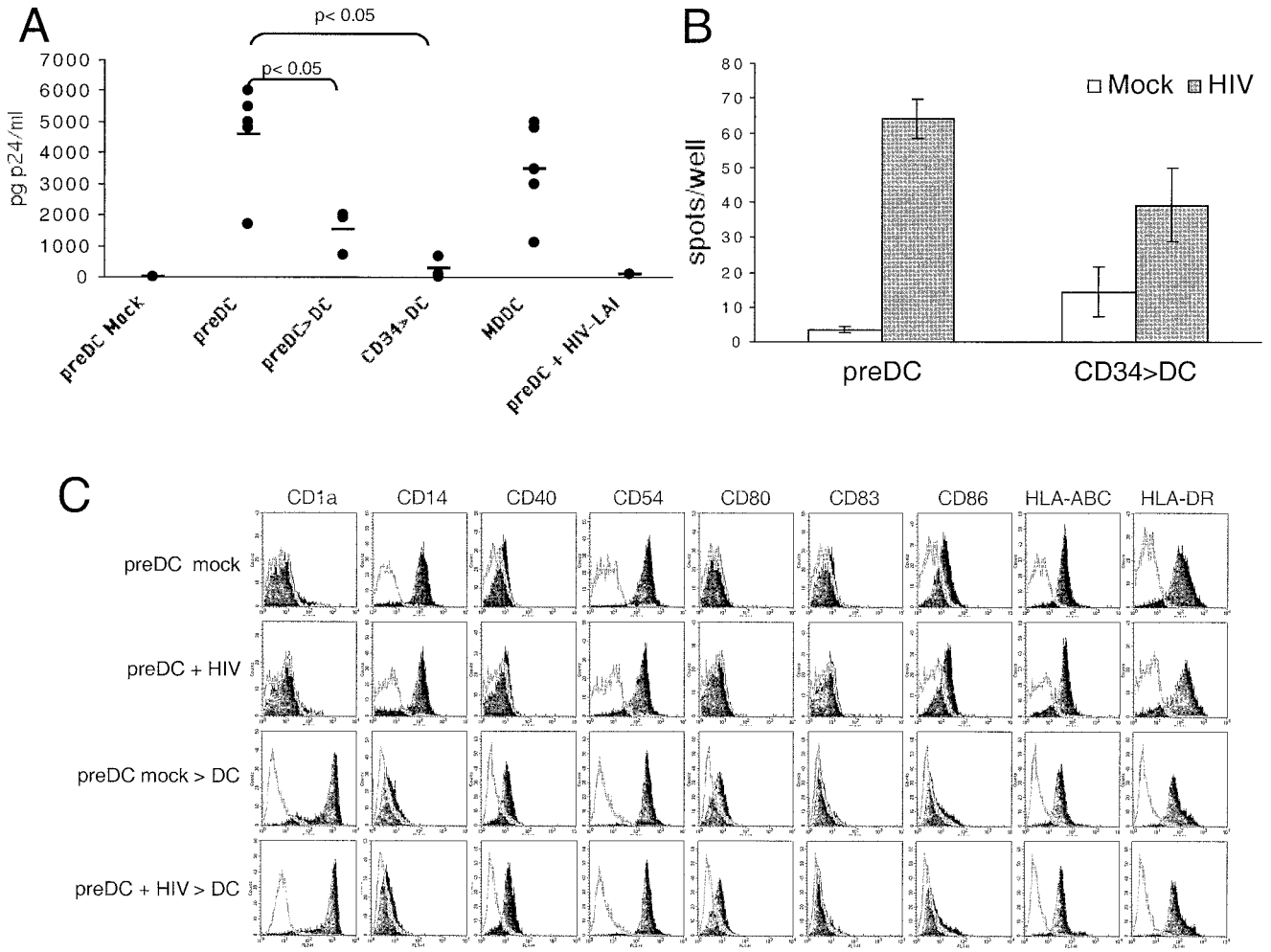

FIG. 4. $CD14^+$ DC precursors are infected by HIV_{BAL} and present an HIV-encoded epitope to specific $CD8^+$ T cells. (A) $CD14^+$ DC precursors (preDC) and $CD14^+$ precursor-derived DC (preDC>DC) or $CD34^+$ progenitor-derived DC (CD34>DC), as well as DC obtained from circulating monocytes (MDDC), were cultured with macrophage-tropic HIV_{BAL}. As controls, preDC were mock treated (preDC Mock) or infected with T-cell-tropic HIV_{LAI} (preDC + HIV-LAI). After 5 days, supernatant HIV-Gag levels were measured using a p24-specific ELISA. The points represent the p24 level in each experiment, and the horizontal bars represent the mean of the data. (B) HLA-$A2^+$ $CD14^+$ DC precursors (preDC) and $CD34^+$ progenitor-derived DC (CD34>DC) were either mock treated (white bars) or infected with HIV_{BAL} (gray bars). After 7 days, the cells were washed, and 2×10^4 cells/well were cultured with a $Pol_{476-484}$-specific HLA-A2-restricted $CD8^+$ T-cell line (T cell:APC ratio of 1:1). The number of IFN-γ-secreting T cells was measured by enzyme-linked immunospot assay and is expressed as the mean ± standard deviation of triplicate tests, representative of three different experiments. (C) Cell surface phenotypic analysis of mock-treated (preDC mock) or HIV-infected $CD14^+$ DC precursors (preDC + HIV). Three days after infection or mock treatment, the cells were cultured for 3 days in GM-CSF–IL-4–TGF-β, and their differentiation into immature DC was assessed by monitoring the expression of different markers. White histograms represent isotype controls, and specific labeling is shown in gray. The data are representative of two different donors.

were permissive to DV infection, but the cellular response in terms of TNF-α and IL-10 synthesis and cell differentiation was different.

$CD14^+$ DC precursors are permissive to HIV infection and present an HIV-encoded epitope to specific $CD8^+$ T cells. We compared preDC and DC with respect to HIV-1 infection, which, like DV, recognizes DC-SIGN as a coreceptor (10, 43) but does not induce DC maturation (14, 22). PreDC and DC generated either from the $CD14^+$ precursors, from $CD34^+$ progenitors, or from adult monocytes were infected with HIV_{BAL}, and viral replication was monitored by measuring the level of Gag p24 protein in the culture supernatant. Figure 4A summarizes p24 concentrations obtained from the HIV-infected APC derived from different donors. PreDC were reproducibly permissive to HIV_{BAL} infection and produced 3- and 10-times-higher p24 levels than DC derived from either preDC or $CD34^+$ progenitors, respectively. The viral p24 production from preDC was not significantly higher than that obtained from monocyte-derived DC. As a control, no p24 was detected from preDC when cultured with the T-cell-tropic strain $HIV\text{-}1_{LAI}$ or when mock treated. Table 2 shows that very little p24 was detectable in the preDC supernatants at day 2 for three different donors, but high levels were measured at day 5, indicative of productive HIV infection. We next compared infected preDC and DC for their ability to present HIV-derived epitopes to $CD8^+$ T cells. HLA-$A2^+$ preDC and DC derived

TABLE 2. Time-dependent production of HIV Gag p24 protein[a]

Donor-strain	Amt of p24 (pg/ml)	
	Day 2	Day 5
Donor A-BAL	321	4,845
Donor B-BAL	379	6,030
Donor C-BAL	42	5,495
Donor A-LAI	113	107

[a] HIV_{BAL} or HIV_{LAI} (at a concentration of 50 ng Gag p24 protein) was added to 10^6 preDC derived from three different donors (A, B, and C), and after 3 h, the cells were washed and cultured. Two days and 5 days afterwards, the concentration of p24 was determined by ELISA.

from the same donor $CD34^+$ progenitors were infected with HIV_{BAL} and cultured with an HLA-A2-restricted Pol-specific $CD8^+$ T-cell line derived from an HIV^+ patient (28). Figure 4B shows that the number of IFN-γ-secreting T cells was higher for infected preDC than for DC; in addition, the difference between mock-treated and HIV-infected cells was greater for preDC. Similar results were obtained using a $CD8^+$ T-cell line specific for the Gag HIV epitope (data not shown). Figure 4C shows that HIV infection did not trigger DC differentiation of preDC, and no apparent phenotypic difference was discernible between infected and mock-treated preDC. HIV infection did not alter the capacity of preDC to convert to DC in the presence of GM-CSF–IL-4–TGF-β, and no overt phenotypic differences were apparent between DC derived from infected or mock-treated preDC (Fig. 4C). HIV-infected preDC or DC did not trigger IL-10 production (data not shown). Thus, $CD14^+$ precursor DC are permissive to macrophage-tropic HIV-1 infection and replication and are capable of presenting HIV-derived epitopes to $CD8^+$ T cells without altering cell phenotype nor affecting their capacity to convert to DC.

DISCUSSION

Cells expressing CD14, DC-SIGN, factor XIIIa, TRANCE, RANK, and sialoadhesin were generated from $CD34^+$ progenitors in the presence of M-CSF. Expression of these and other markers suggests a close relationship with cells residing in the interstitial space of dermis and mucosa, which have been referred to as dendrocytes (18), dendrophages (33), or $CD14^+$ DC (43). We have called these in vitro-generated cells preDC, since in the presence of GM-CSF–IL-4–TGF-β, the cells converted into $CD1a^+$ $CD14^-$ immature DC that responded to LPS by maturation into potent T-cell stimulators. Direct stimulation of preDC by LPS does not induce this activity. In this regard, preDC resemble dermal $CD14^+$ cells, which have been shown to be poor T-cell stimulators (26, 32) but can convert into DC in the presence of GM-CSF (25). Monocytes are likewise myeloid DC precursors, but the in vitro-generated preDC differ from monocytes by expression of markers such as factor XIIIa and DC-SIGN and by the fact that monocytes require more than 3 days for optimal DC differentiation (37). Another potential difference is that the cytokine combination of GM-CSF–IL-4–TGFβ induces differentiation into interstitial-type and not Langerhans-type DC, as observed for monocytes (13). However, GM-CSF–TGF-β triggers conversion of preDC into Langerhans-type DC (J-.B. Barbaroux et al., submitted).

The strategic position of interstitial $CD14^+$ cells below the basal membrane, their close association with endothelial cells, mastocytes, and T cells, and their capacity to rapidly differentiate into DC make it probable that the cells play an important role in the defense against infectious agents. This hypothesis is supported by the finding that interstitial $CD14^+$ cells carry the C-type lectin DC-SIGN (23, 43) or *N*-acetylgalactosamine-specific C-type lectin (19, 44), implicated in viral pathogen entry (10, 31, 41–43). We found that preDC expressed DC-SIGN as well as the C-type lectin mannose receptor and the I-type lectin sialoadhesin, and we compared preDC and DC susceptibility to DV and HIV-1 infection.

DV is a mosquito-borne arbovirus that causes significant morbidity and mortality worldwide (15) and consists of four antigenically related serotypes. It is thought that virus-specific, nonneutralizing antibodies or serotype-cross-reactive T cells generated during a first exposure enhance subsequent viral infections with different serotypes (36). Antibody-mediated enhancement of infection (16) accompanied by high levels of T-cell cytokines may participate in the pathology of dengue hemorrhagic fever (35). Interactions between DV and monocyte-derived DC have been characterized; DV infects and replicates in monocyte-derived DC and provokes their maturation (20, 27). Furthermore, it has been shown that DC-SIGN plays a major role in DV entry (31, 42). Little is known about the $CD14^+$ interstitial cells in DV infection, although this may be relevant as the mosquito bite reaches the dermis to draw blood. We found that DV serotype 2 infects and replicates in preDC, in contrast to monocytes, which are inefficiently infected by DV in the absence of antibody-dependent enhancement (8, 21). However, preDC yielded consistently fewer infectious DV particles than DC. Although cell apoptosis was not directly measured, CD14 and CD1a marker expression on the total, ungated cell population was used as an indicator of cell death. We found that at day 2, when virion production was assessed, the percentage of live, labeled cells was identical for preDC and $CD14^+$ precursor-derived DC, making it unlikely that DV-induced cell death is responsible for lower virion yields. Furthermore, the viral yield from preDC was as high as that reported for DC derived from adult monocytes (20, 27) and 10-fold higher than that reported for monocyte-derived macrophages (6). These data suggest that not only Langerhans cells (46) or interstitial DC (20, 27, 31, 42) but also interstitial $CD14^+$ cells participate in DV infection. DV yield from preDC, together with the large number of interstitial $CD14^+$ cells in the skin, compared to Langerhans cells and interstitial DC, implies that these cells may play a significant role in DV replication early after infection.

As expected, infected DC produced TNF-α but no IL-10 (20, 27), and the TNF-α is likely to be responsible for the observed DC maturation (29). In contrast, preDC secreted IL-10 but no detectable TNF-α. It would be interesting to test whether other DV serotypes or primary isolates have a similar effect with regard to cytokine production by preDC and DC. In this regard, DV serotype 2 has been shown to induce TNF-α levels by monocyte-derived DC that are up to twofold higher than those induced by other serotypes, suggesting interserotype variability (20). Also, it may be important to test different MOI and

investigate whether opsonized DV induces a different cytokine response or simply amplifies the observed cytokine release due to higher infectivity. The anti-inflammatory effects of IL-10 may explain the lack of TNF-α production and lack of CD83 expression on preDC (30), since we have found that IL-10 released by LPS-treated preDC prevents CD83 expression and differentiation into T-cell-stimulatory APC (W.-H. Kwan et al., unpublished data). In addition to its anti-inflammatory effects, IL-10 up-regulates CD16 and CD64 Fcγ receptor expression (30) and could thus enhance secondary DV infection through an antibody receptor entry route. In addition, IL-10 modulates T-cell priming and promotes polarization of T cells toward the Th2 type, which favors the humoral immune response.

With respect to HIV, preDC were permissive to HIV_{BAL} and released considerably more viral p24 protein than infected DC derived from preDC or directly from $CD34^+$ progenitors. The amount of p24 was roughly equivalent to that measured in cell supernatants of monocyte-derived DC. The reason for this difference is not clear, but it may be related to different levels of receptor expression and/or virus replication. It would be interesting to compare p24 production between monocyte-derived $CD14^+$ DC-$SIGN^+$ cells and monocyte-derived DC. We are currently determining the conditions to obtain $CD14^+$ DC-$SIGN^+$ cells from monocytes. Infected preDC were efficient in presenting HIV-derived epitopes to a $CD8^+$ T-cell line derived from an HIV-infected patient (28). As for most memory T-cell lines, activation of this T-cell line reflects more the number of peptide-loaded major histocompatibility complexes than expression of costimulatory factors (CD80, CD86) necessary for naive T-cell priming. PreDC and $CD34^+$ progenitor-derived DC express identical levels of HLA-A2; thus, the T-cell activation is correlated to the number of HIV-epitope-loaded major histocompatibility complex class I complexes. This number is likely to be higher in preDC, since HIV more efficiently infected these cells. HIV-1 infection neither inhibited preDC from converting into DC nor triggered their maturation, as shown previously for DC (14). Taken together, this suggests that interstitial $CD14^+$ cells may, on one hand, activate local memory T cells and enhance an antiviral immune response and, on the other hand, enhance viral spread to T cells by converting to DC and shuttling virions to draining lymph nodes. In the same context, it is interesting that Nef-expressing or CD40L-activated macrophages release a factor that renders resting T lymphocytes permissive to HIV-1 infection in the presence of B cells (40). Infected interstitial $CD14^+$ cells may also participate in rendering resting T cells permissive to HIV.

DV and HIV infections differ in their immunological outcome. DV triggers a strong antiviral immune response, while HIV infection is characterized by a slow, latent response. The differences in early cell target infectivity together with different cellular responses may help to explain these phenomena. The latent anti-HIV immune response may be in direct relation with the efficient infection of the long-lived preDC and its inability to induce DC maturation. The rapid anti-DV immune response may be associated with efficient infection of DC and with induction of TNF-α production and DC maturation. DC maturation may lead to a rapid antiviral immune response, which may contribute to asymptomatic infections in about 70% of primary cases. In addition, release of IL-10 by DV-infected preDC may help to balance the cytotoxic response with production of DV-specific antibodies. It is plausible that upon secondary DV infection, opsonization may enhance DV infection of preDC and concomitantly IL-10 production. Interestingly, a recent study showed that patients with secondary DV infections and dengue hemorrhagic fever had significantly higher serum IL-10 levels than control patients (34).

Interstitial $CD14^+$ cells have eluded rigorous studies in part because of low cell recovery from clinical biopsies, and one of the underlying reasons may be their intrinsic propensity to convert into DC. $CD34^+$ progenitor-derived preDC could be used as an in vitro model for interstitial $CD14^+$ cells, and the ability to differentiate the cells into DC has allowed us to compare the two cell types with respect to pathogen infection and antipathogen immune response. The results show that preDC can be viral targets, present viral epitopes, and undergo different cell responses that may affect viral dissemination and modulate the antiviral immune response.

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