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The Maturation of Dendritic Cells Results in Postintegration Inhibition of HIV-1 Replication

Youssef Bakri,²*,²† Cécile Schiffer,²*, Véronique Zennou,‡ Pierre Charneau,‡ Edmond Kahn,† Abdelaziz Benjouad,³*, Jean Claude Gluckman,⁎ and Bruno Canque³*

Maturation of dendritic cells (DC) certainly play an important role in the natural history of HIV infection (1). There is evidence that Langerhans cells (LC) from genitinal or rectal epithelia are among the first targets of HIV (2–4), and could be responsible for both virus transport to draining lymph nodes (5) and preferential transmission of CCR5-dependent R5 strains (6–8). DC from lymphoid tissues (e.g., tonsils, adenoids, lymph nodes, Peyers patches) of chronically infected subjects are frequently involved in p55 S100 CD68 multinucleated giant cells (9–12) or closely associated with HIV- or SIV-infected T lymphocytes (13–17), while LC from the cervix and vaginal lamina propria could represent a virus reservoir in vivo (18). Accordingly, DC and LC isolated from the blood, skin, or genital tract, or differentiated in vitro from CD34+ hemopoietic progenitor cells (HPC) or from monocytes, express CD4 and HIV coreceptors, CCR5 and CXCR4, and are permissive to HIV (8, 19–33).

In vitro studies have disclosed the high complexity of HIV interactions with DC and their tight regulation by extrinsic activation/maturation signals. CCR5 immature DC (iDC) differentiated from CD34+ HPC, or freshly isolated from the skin, have been shown to express low to undetectable membrane CXCR4 and to selectively replicate R5 viruses (8, 34, 35). Maturation induced by short-term culture of epidermal LC, or by adding monocyte-conditioned medium (MCM) or trimeric CD40 ligand (CD40LT) to in vitro differentiated DC, results in decreased CCR5 expression and CXCR4 up-regulation, but increased permissivity to both X4 and R5 strains (8, 34, 35). However, at variance with CD4+ T lymphocytes (36) that require activation signals to complete HIV reverse transcription and support virus replication, virus production appears 10- to 100-fold lower in iDC than in mDC (34, 35). The mechanisms underlying this apparently paradoxical phenomenon are poorly characterized, although several nonexclusive hypotheses have been proposed, ranging from inhibition of virus entry due to β-chemokine production by MDC (37) to inhibition of viral reverse transcription (26, 38) to the incapacity to drive HIV long-terminal repeat (LTR) transcription (39). To approach this question, we examined in this study the influence of the maturation of DC on HIV entry and reverse transcription, nuclear import of the viral preintegration complex (PIC), integration of viral DNA, and HIV LTR transcription.

Materials and Methods

Cells

Cytophereses from healthy platelet donors were collected according to institutional guidelines (Site Transfusional Pitie-Salpetriere, Paris, France).
PBMC were isolated by Ficoll-Paque (Pharmacia, Uppsala, Sweden) centrifugation. To generate DC from monocytes, PBMC (3.3 × 10^6/ml) were seeded in six-well plates (ATGC, Noisy le Grand, France) (1 × 10^6/well) in RPMI 1640 and 10% heat-inactivated AB serum (Life Technologies, Paisley, UK). After 1 h at 37°C in humidified 5% CO₂, nonadherent cells were removed, and adherent cells were cultured for 5 days in RPMI 1640, 10% FCS (Life Technologies, 1% glutamine, 1% antibiotics (penicillin-streptomycin-neomycin, 50 μg/ml) (Life Technologies, Paisley, UK). After 1 h at 37°C in humidified 5% CO₂, nonadherent cells were removed, and adherent cells were cultured for 5 days in RPMI 1640, 10% FCS (Life Technologies, 1% glutamine, 1% antibiotics, with 10 ng/ml GM-CSF and IL-4 (gift of Schering-Plough, Kenilworth, NJ). Cells were fed on day 2 by removing one-third of supernatant and adding fresh medium with full doses of cytokines. CD1a⁺CD83⁺ iDC represented >90% of nonadherent cells (data not shown) on culture day 5, with <1% contaminating CD3⁺ T lymphocytes. DC were induced to mature by further culturing day 5 iDC for 72 h with the same cytokines plus CD40LT (250 ng/ml; gift of Immunex, Seattle, WA) or MCM (20% final) (40), resulting then in CD83⁺ mDC.

**Flow cytometry cell surface marker analysis**

Cells were incubated for 30 min at 4°C with mAbs (1:100 final unless specified) in PBS, 2% FCS, washed, and analyzed with a FACSSCalibur (Becton Dickinson, San Jose, CA). DC were examined with the following FITC- or PE-conjugated mAbs: CD4-FITC (BB731, Becton Dickinson, San Jose, CA); CD8-FITC (Becton Dickinson, San Jose, CA); and Isotype-matched FITC- and PE-conjugated irrelevant control mAbs were from Immunotech and Becton Dickinson, respectively.

**HIV-1 strains and HIV-1 expression plasmids**

R5 HIV-1BaL (41) (gift of B. Asjo, Bergen, Norway) was produced in monocyte-derived macrophages, as described (42). X4 HIV-1AD (43) was purchased from Diagnostics Pasteur (Marseille la Coquette, France). Supernatants were titrated in P4-CCR5 indicator cells, as described (44).

T lymphocyte blasts (5 × 10^6/ml) were generated by culturing PBMC in RPMI 1640, 10% FCS, 1% glutamine, 1% antibiotics, with 1 μg/ml PHA (Sigma, St. Louis, MO) for 3 days, and then with 100 μg/ml IL-2 (gift of Chiron, Amsterdam, The Netherlands).

**Quantitation of virus production**

**Nuclear import and integration of HIV PIC**

Southern blot analysis was used to assess full-length nontransduced linear HIV DNA, as well as nuclear 1 and 2 LTR circles and total viral DNA in HIV-exposed DC (51) (see also Fig. 3A). Cells (5 × 10^6/ml) were exposed for 96 h to 1.5 μg p24 equivalent of pNL-3 or pNL(AD) clones, or of VSV-G-pseudotyped recombinant pNL-3-GFP. Each virus contained the continuous presence of 1 μM saquinavir (Roche). They were lysed, and total DNA was extracted by the organic method (Wizard Genomic DNA, Promega, Madison, WI). DNA extracts were then incubated overnight at 37°C under agitation in 20 μl of a digestion solution in distilled water, containing 1 μl MseI (15 U), 1 μl XhoI (20 U), and 2 μl DpnI (20 U) restriction endonucleases; 2 μl 10 × TP4; and 2 μl BSA (1 mg/ml) (all from Biolab, Beverly, MA). DpnI digests the bacteria-derived plasmid DNA, while MseI digests the genome positions 2658 and 4590, and XhoI at position 8896. Of note, de novo synthesized viral DNA is resistant to cleavage by DpnI. XhoI was used to minimize transfer bias due to the large size of circular fragments. This procedure generates a 1.9-kb MseI internal fragment, which corresponds to the total amount of viral DNA; a 2.6-kb band corresponding to the distal 5′ MseI fragment, specific of full-length linear HIV DNA known not to be transcribed in the nucleus and to be associated with the nuclear envelope (51); and two bands of 2.8 and 3.4 kb that represent 1 and 2 LTR nuclear circles, respectively.

Equivalent amounts of total DNA from T lymphocytes, iDC, and mDC were then electrophoresed overnight in 0.7% agarose, denatured for 30 min in 0.4 N NaOH, capillary transferred, and fixed onto a Hybond N⁺ membrane by incubation in 0.4 N, 1.5 N, and 0.4 N NaOH solutions, respectively. To detect HIV DNA fragments, hybridization was performed by incubation in 0.4 N, 1.5 N, and 0.4 N NaOH solutions, respectively. To detect HIV DNA, as well as nuclear 1 and 2 LTR circles and total viral DNA in HIV-exposed DC (51) (see also Fig. 3A). Cells (5 × 10^6/ml) were exposed for 96 h to 1.5 μg p24 equivalent of pNL-3 or pNL(AD) clones, or of VSV-G-pseudotyped recombinant pNL-3-GFP. Each virus contained the continuous presence of 1 μM saquinavir (Roche). They were lysed, and total DNA was extracted by the organic method (Wizard Genomic DNA, Promega, Madison, WI). DNA extracts were then incubated overnight at 37°C under agitation in 20 μl of a digestion solution in distilled water, containing 1 μl MseI (15 U), 1 μl XhoI (20 U), and 2 μl DpnI (20 U) restriction endonucleases; 2 μl 10 × TP4; and 2 μl BSA (1 mg/ml) (all from Biolab, Beverly, MA). DpnI digests the bacteria-derived plasmid DNA, while MseI digests the genome positions 2658 and 4590, and XhoI at position 8896. Of note, de novo synthesized viral DNA is resistant to cleavage by DpnI. XhoI was used to minimize transfer bias due to the large size of circular fragments. This procedure generates a 1.9-kb MseI internal fragment, which corresponds to the total amount of viral DNA; a 2.6-kb band corresponding to the distal 5′ MseI fragment, specific of full-length linear HIV DNA known not to be transcribed in the nucleus and to be associated with the nuclear envelope (51); and two bands of 2.8 and 3.4 kb that represent 1 and 2 LTR nuclear circles, respectively.

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amounts of unintegrated linear and circular HIV DNA species: DNAi (%)) = 100 − [linear DNA (%) + circular DNA (%)].

HIV LTR transcription assays

The iDC (1 × 10^6/ml) were exposed for 48 h to 0.75–1.5 μg/ml p24 equivalent of VSV-G-pseudotyped recombinant pNL4-3.GFP.E₂ or pNL4-3.ΔU3.CMV.GFP.E₂, or to 1 μg/ml p24 equivalent of pNL4-3.Luc.R.E₂, washed, cultured in the presence of GM-CSF and IL-4 with or without CD40LT or MCM, and analyzed 48 or 96 h after induction of maturation. GFP fluorescence was analyzed with a FACSCalibur and quantified as follows (MFI, mean fluorescence intensity): GFP activity = (MFI GFP^+ cells/MFI GFP^- cells) × %GFP^+ cells. Firefly luciferase activity in cell lysates was assayed with a commercial kit (Luciferase assay system; Promega) and a 1450 Microbeta Plus luminometer, and expressed as arbitrary luminescence U/10^6 cells.

Confocal microscopy

Cells were washed with PBS and cytospun onto glass slides, which were dried before 10-min fixation at 20°C in PBS, 3% paraformaldehyde, and permeabilized with PBS, 0.05% saponin, 0.2% BSA, 0.5% AB serum. Cells were stained with 1/100 diluted polyclonal goat anti-NF-κB p50 and p65 Abs, or rabbit Abs against NF-κB p52, RelB, c-Rel, or anti-SP1 and SP3 transcription factors. Normal rabbit or goat sera (Dako, Carpenteria, CA) were used as controls. Staining was developed with biotinylated swine anti-goat, anti-mouse, anti-rabbit multilink Abs (Dako) diluted 1/50, followed by tetramethylrhodamine isothiocyanate-conjugated streptavidin (Immunotech) diluted 1/200. Slides were mounted in fluorescent-mounting medium (Dako). Confocal laser-scanning microscopy and fluorescence analysis were performed using a SARASTRO CLSM1000 confocal microscope (Molecular Dynamics, Sunnyvale, CA). Excitation was obtained by an argon laser filtered at 514 nm, which ensures low background light and tetramethylrhodamine isothiocyanate emission. The selected dichroic filter was DF 530 nm. To avoid excitation noise at emission, fluorescence acquisition was performed using a 550-nm highpass filter in 256 × 256 pixel matrices, and a 50-μm pinhole size. Laser power was set to values ranging from 8 to 12 mW, and the PM detector was set at values ranging from 1000 to 1200 V (sensitivity adjustments). Images at 0.25- or 0.5-μm pixel size were obtained at ×40 magnification, 1 numerical aperture, and analyzed as reported (52).

Results

We have shown that iDC differentiated from CD34^+ HPC support R5 but not X4 HIV-1 strain replication, and that inducing DC maturation by CD40 ligation or use of MCM results in a 10- to 100-fold decrease in R5 HIV-1 Ba.L replication (34). Because monocyte-derived DC (MDDC) represent a useful tool to study the interactions of HIV with DC (35), preliminary experiments were performed to assess their capacity to support virus replication whether as iDC or as mDC.

We first examined HIV coreceptor expression of MDDC (Fig. 1): iDC recovered on day 5 from monocytes cultured with GM-CSF and IL-4 were cultured for another 72 h with or without CD40LT or MCM, before FACS analysis. The iDC were CD1a^−CD83^+ and expressed both CCR5 and CXCR4, which differs from CD34^+ HPC-derived iDC that mainly lack membrane and cytoplasmic CXCR4 (34). Of note, a minor population of CXCR4^+CD83^− mDC was always present in these cultures. In line with other reports (4, 21), adding MCM or CD40LT to DC (referred to thereafter as DC_MCM or DC_CD40LT, respectively) induced similarly high levels of membrane CD83 and CXCR4 by mDC, whereas CCR5 expression was still low on DC_CD40LT, but no longer detectable on DC_MCM, which suggests that entry of R5 viruses might be influenced by the conditions under which DC are led to mature. Since MCM contains significant levels of CCR5 ligands RANTES, macrophage-inflammatory protein (MIP)-1α,
whether they had been exposed to HIV-1Ba-L or HIV-1 LAI, which
II). The iDC harbored then low but similar HIV DNA amounts
ing from strong-stop to full-length proviral DNA (Fig. 2 and Table
PCR with R-U5 primers, which detects all HIV DNA species rang-
entry was evaluated by semiquantitative endpoint dilution nested
DC CD40LT were exposed to HIV-1 Ba-L or HIV-1 LAI as above, fol-
CXCR4 up-regulation in DCCD40LT was associated with strongly
had been added or not, were sequentially assessed for up to 96 h
(53). DC MCM also exhibited decreased CCR5 transcription in the face of DC MCM could result both from decreased CCR5 transcription and ligand-mediated endocytosis. DC MCM also exhibited limited decrease of CD4 expression. In parallel, culture day 5 iDC were exposed to 500 TCID of R5 HIV-1Ba-L or X4 HIV-1LAi, and cultured for another 48 h with GM-CSF and IL-4 to allow virus dissemination in the cultures; they were then washed, and HIV RNA copy numbers in supernatants of cultures, in which CD40LT had been added or not, were sequentially assessed for up to 96 h (Table I). Under these conditions, like their CD34+ HPC-derived counterparts, iDC deriving from monocytes replicated more efficiently HIV-1Ba-L than HIV-1 LAi, differences in viral RNA copy numbers averaging 1.8 log; adding CD40LT led to ~0.7-log reduction of HIV-1 Ba-L RNA copy numbers, but to no significant change in HIV-1 LAi production on day 4 postinduction of maturation.

Altogether, these data demonstrate that MDDC can substitute for DC differentiated from CD34+ HPC as a model to study the interactions of HIV with DC.

The permissivity of mDC to R5 or X4 viruses depends on the maturation conditions
To assess their permissivity to HIV, culture day 8 iDC, DC MCM, or DC CD40LT were exposed to HIV-1Ba-L or HIV-1LAi as above, followed by 48-h culture under the same conditions, after which virus entry was evaluated by semiquantiative endpoint dilution nested PCR with R-U5 primers, which detects all HIV DNA species ranging from strong-stop to full-length proviral DNA (Fig. 2 and Table II). The iDC harbored then low but similar HIV DNA amounts whether they had been exposed to HIV-1Ba-L or HIV-1LAi, which indicates that, at variance with CD34+ HPC-derived DC (34), both R5 and X4 viruses efficiently entered into monocyte-derived iDC. CXCR4 up-regulation in DC CD40LT was associated with strongly increased HIV-1LAi entry, whereas HIV-1Ba-L entry did not change or increased marginally despite their reduced CCR5 expression. Inducing DC maturation with MCM also resulted in increased permissivity to HIV-1LaI, although DC MCM usually harbored less HIV DNA than DC CD40LT. Finally, loss of membrane CCR5 by DC MCM was associated with reduced permissivity to HIV-1 Ba-L (Fig. 2B and Table II).

These data indicate that the permissivity of DC to X4 and R5 HIV-1 strains not only depends on their maturation stage, but also on the signals that trigger maturation.

**DC maturation does not inhibit HIV reverse transcription**
We next examined the influence of DC maturation on viral reverse transcription. Culture day 8 iDC and DC CD40LT were exposed as previously to HIV-1LaI or HIV-1Ba-L, and analyzed 48 h postinfection by nested PCR. Of note, due to their limited permissivity to the virus, iDC were exposed to 500 or 104 TCID to allow for more accurate quantification of late DNA products. Reverse-transcription efficiency (RE) was then assessed as the difference between the LET obtained by LTR-gag PCR, which amplifies late DNA products formed after the second template switch, and the LET of R-U5 PCR, which amounts to total HIV DNA (Table III): this showed that although REs noted with HIV-1Ba-L were on average ~4-fold higher than with HIV-1LAi, iDC and DC CD40LT had a comparable capacity to support reverse transcription. Similar results were obtained when cells were analyzed 96 h instead of 48 h postinfection, which rules out the possibility of delayed reverse transcription in either iDC or mDC (data not shown). Similar REs were found in DC CD40LT infected with HIV-1LAi or HIV-1Ba-L, than in DC MCM infected with the same strains, indicating in addition that reverse transcription was independent of the DC maturation conditions (data not shown).

**FIGURE 2.** Nested PCR detection of total HIV DNA in iDC and mDC. Culture day 8 iDC (A), DC CD40LT, and DC MCM (B) were exposed to HIV-1Ba-L or HIV-1LAi, and cultured for 48 h with GM-CSF and IL-4, with or without adding CD40LT or MCM. PCR lysates were then prepared, and HIV-1 DNA was amplified by using LTR R-U5 primers. PCR analysis of the β-globin gene was used as amplification and DNA content control. Control amplification was also performed in lysates from uninfected DC and mock infected (data not shown). Endpoint dilution analysis was performed as indicated in Table II; figures indicate the inverse of dilutions. Results of one experiment of two (A) or five (B).
We also examined HIV reverse transcription in PHA/IL-2-activated T lymphocytes exposed to HIV-1 Ba-L or HIV-1 LAI under the same conditions as the DC. Comparable HIV DNA amounts were found in R-U5- and LTR-gag-infected T lymphocytes, which indicates that most reverse transcripts ended up as late DNA products. LTR-gag DNA represented then the equivalent of 45% of total HIV DNA in HIV-1 Ba-L-infected T lymphocytes (Table III).

Thus, in the same manner as macrophages (54), but in contrast to T lymphocytes, DC have a higher capacity to support reverse transcription of R5 than of X4 HIV-1 strains independently of their maturation stage.

DC maturation does not interfere with HIV DNA nuclear import or integration

Because the above data demonstrated that the decreased capacity of mDC to support HIV replication could not be accounted for by inhibition of reverse transcription, we next examined the effect of DC maturation on HIV DNA nuclear import and/or integration. This was performed by Southern blot detection of HIV DNA products using a recently described method based on a particular restriction cleavage and probe strategy (Fig. 3A), which allows for the simultaneous detection of total (1.9 kb), nontranslocated linear (2.6 kb) HIV DNA, and unintegrated nuclear 1 and 2 LTR circles (2.8 and 3.4 kb, respectively) (51). The iDC and DCCD40LT were exposed for 96 h to a high amount of R5 pNL(AD8) molecular clone, in the continuous presence of saquinavir to restrict the afferent phase of HIV replicative cycle, before DNA was prepared, digested with MscI and XhoI, and hybridized with a probe overlapping the 5′ MscI site. Under these conditions, the intracellular viral DNA pattern noted in iDC and mDC was comparable, with 1 LTR circles, linear and integrated HIV DNA representing 45%, 20%, and 25% of total viral DNA, respectively (Fig. 3B). Of note, iDC constantly harbored higher amounts of 2 LTR circles than mDC. Similar results were obtained when cells were exposed to virus for 48 h only and analyzed after 96 h as previously, excluding thus minor differences between iDC and mDC. The same analysis showed that PHA/IL-2-activated T lymphocytes, which had been infected under the same conditions as the DC, essentially lacked linear DNA, with 1 LTR circles and integrated HIV DNA representing then 40% and 60% of total HIV DNA, respectively (Fig. 3B). Thus, independently of the maturation stage, HIV nuclear import and integration proceeded less efficiently in DC than in PHA/IL-2-activated T lymphocytes. Finally, the fact that comparable results were found in DC and T lymphocytes exposed to X4 pNL4-3, R5 pNL(AD8), or VSV-G-pseudotyped viruses (data not shown) indicated that coreceptor usage or the mode of virus entry did influence neither nuclear import nor integration of viral DNA.

DC maturation down-modulates HIV transcription

Because the above data indicated that DC maturation did not interfere with the afferent phase of HIV replicative cycle, we next examined whether this could affect HIV LTR transcription. To avoid signaling via CXCR4 or CCR5 (64), iDC were exposed for 48 h to VSV-G-pseudotyped pNL4-3-GFP virus, a high amount of which was used to ensure high transduction efficiency. The cells were then cultured further for 96 h under the standard condition or with CD40LT. At that time, GFP activity determined by FACS averaged 319 ± 258 arbitrary U in iDC relative to 187 ± 169 for DCCD40LT (p = 0.04), which corresponded to 46 ± 11% reduction (Fig. 4A). Because these data strongly suggested that maturation altered the capacity of DC to drive HIV LTR, we next performed similar experiments using recombinant vectors expressing the firefly luciferase, which allows more accurate quantification of the phenomenon. The iDC were exposed as previously to VSV-G-pseudotyped pNL4-3-Luc virus, and cultured for 96 h with or without CD40LT or MCM before assaying luciferase activity. Luciferase activity in DCCD40LT and DCMCM represented then 25 ± 10% and 46 ± 13%, respectively, of that found in iDC (p = 0.01) (Fig. 4B). No GFP or luciferase activity was detected when experimental infections were performed in the presence of nevirapine, which excludes the possibility of cell pseudotransduction (Fig. 4B, and data not shown). Altogether, these findings demonstrate that postintegration-negative regulatory mechanisms occurring at

Table III. Analysis of the efficiency of HIV reverse transcription in DC and T lymphocytes

<table>
<thead>
<tr>
<th>HIV-1 Strain</th>
<th>iDC</th>
<th>CD40LT</th>
<th>T lymphocytes</th>
<th>RE: p (CD40LT vs iDC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAI LET (R-U5)</td>
<td>0.7 ± 1.1</td>
<td>1.3 ± 0.5</td>
<td>3.75 ± 0.25</td>
<td>NS</td>
</tr>
<tr>
<td>LAI LET (LTR-gag)</td>
<td>0.2 ± 0.7</td>
<td>2.7 ± 0.8</td>
<td>3.83 ± 0.17</td>
<td>NS</td>
</tr>
<tr>
<td>LAI RE</td>
<td>1.0</td>
<td>0.2</td>
<td>83.2</td>
<td></td>
</tr>
<tr>
<td>LAI % efficiency</td>
<td>79</td>
<td>10.0</td>
<td>44.7</td>
<td></td>
</tr>
<tr>
<td>Ba-L LET (R-U5)</td>
<td>2.4 ± 0.3</td>
<td>2.8 ± 1.1</td>
<td>2.45 ± 0.00</td>
<td>NS</td>
</tr>
<tr>
<td>Ba-L LET (LTR-gag)</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.8</td>
<td>0.35 ± 0.00</td>
<td>NS</td>
</tr>
<tr>
<td>Ba-L RE</td>
<td>0.06</td>
<td>0.05</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Ba-L % efficiency</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*On culture day 8, iDC, DCCD40LT, and PHA/IL-2-activated T lymphocytes, were exposed to HIV-1 Ba-L or HIV-1 LAI. PCR lysates were prepared 48 h after infection, and HIV-1 DNA was analyzed using LTR R-U5 and LTR-gag primers. Endpoint dilution analysis was performed as indicated in Table II. Similar serial dilutions of cell lysates were performed for all samples to allow comparison between HIV DNA amounts detected by LTR R-U5 and LTR-gag PCR. RE was computed as LET (LTR-gag) – LET (R-U5); the percentage of efficiency corresponds to the antilog of the RE expressed as a percentage. Statistical analyses were performed using the paired Student t test to compare infections with HIV-1 Ba-L vs HIV-1 LAI or the unpaired test when comparing infections of iDC vs DCCD40LT. NA, Not applicable.
the transcriptional level are responsible for the decreased capacity of mDC to support HIV replication.

The specificity of this phenomenon was examined by investigating whether maturation of DC also affected the capacity to drive another viral promoter: iDC were exposed as before to a VSV-G-pseudotyped self-inactivating HIV vector that contains the GFP gene under the exclusive control of an internal CMV promoter, and pseudotyped self-inactivating HIV vector that contains the GFP gene under the exclusive control of an internal CMV promoter, and another viral promoter: iDC were exposed as before to a VSV-G- 

Discussion

Recent data on the interactions of DC with HIV have reconciled more than a decade of debate (34, 35, 38) by clearly demonstrating that discrepant findings regarding DC permissivity to HIV were due to different technical conditions leading to heterogeneous DC populations as to both origin and maturation stage. We have shown (34) that CCR5CXCR4low/iDC differentiated from CD34+ HPC are preferentially susceptible to, and support subsequent replication of R5 HIV-1 strains. Upon maturation, CD34+ HPC-derived DC down-modulate CCR5 while acquiring membrane CXCR4, and they become permissive to X4 as well as to R5 viruses. However, in contrast with their immature counterparts, mDC present a postentry block of virus replication, which results in a 10- to 100-fold drop in virus production (34). The mechanisms responsible for this phenomenon are currently unclear, although several explanations have been proposed, ranging from inhibition of HIV reverse transcription to the constitutive incapacity of DC to drive HIV LTR. As an attempt to clarify this point, we comparatively analyzed in this study the major steps of HIV replicative cycle in both iDC and mDC: i.e., virus entry, reverse transcription, nuclear import and integration, and transcription.

We first examined how different maturation signals, provided by CD40 ligation or soluble factors present in MCM, could affect MDDC permissivity to the virus. At variance with iDC differentiated from CD34+ HPC, which express CCR5 but lack CXCR4,
HIV-1 LAI efficiently entered these iDC, although virus production was reduced permissivity to R5 HIV-1. Finally, maturation induced by CD40LT led to CXCR4 up-regulation and facilitated HIV-1 LAI entry in a short-term assay. Maturation induced by MCM or CD40LT was associated with detectable membrane CCR5 on DC MCM was associated with reduced permissivity to R5 HIV-1. Interestingly, DC CD40LT harbored greater R-U5 HIV DNA amounts than DC MCM, suggesting that CD40 ligation may enhance virus entry and/or stimulate early steps of reverse transcription. At variance with CXCR4, CCR5 expression varied according to the maturation conditions: DC CD40LT still expressed low membrane CCR5 and allowed efficient entry of HIV-1 Ba-L, whereas lack of detectable membrane CCR5 on DC MCM was associated with reduced permissivity to R5 HIV-1. Finally, maturation induced by CD40 ligation resulted in 5-fold decrease in HIV-1 Ba-L production, but did not significantly change that of HIV-1 LAI, which is in line with our previous report (34).

Comparative analysis of DC that had been exposed to HIV-1 LAI or HIV-1 Ba-L, as either iDC or mDC showed that maturation had no positive or negative effect on virus reverse transcription. Rather, due to their increased permissivity to HIV, mDC could harbor higher LTR-gag DNA amounts than iDC. These results are at variance with reports showing decreased RE in mDC (26, 63), but the conditions utilized in these reports did not allow actual comparison of iDC vs mDC; for example, they compared infection of T blasts with that of DC MCM by X4 HIV-1 R5, which poorly reverse transcribes in DC (26), or of iDC and DC MCM by HIV-1 Ba-L, the entry of which into the latter cells is restricted (35). Conversely, virus reverse transcription in DC was tightly dependent on HIV coreceptor use. Indeed, like macrophages and in contrast to PHA/IL-2-activated T lymphocytes, DC displayed a lower capacity to support reverse transcription of X4 than of R5 viruses. Whether such differences are due to different signaling through CXCR4 and CCR5 cytoplasmic domains (64) or to the delivery of virus particles to different intracellular compartments is presently unknown. Thus, usage of different coreceptors by different HIV strains may reflect the adaptation to their primary lymphoid and/or myeloid target cells. From a pathophysiological point of view, one may consider that the increased capacity of mucosal macrophages and LC to support reverse transcription of R5 viruses could be responsible for their predominant sexual transmission.

Analysis of the final stages of HIV replicative cycle afferent phase in DC showed that neither nuclear translocation of the viral PIC nor integration of HIV DNA was affected by maturation, although both processes were much less efficient than in PHA/IL-2-activated T lymphocytes. Of interest, identical Southern blot patterns of HIV DNA fragments were noted when DC were infected with X4 HIV-1 LAI, R5 pNL(AD8), or VSV-G-pseudotyped pNL4-3 molecular clones (data not shown), showing that nuclear translocation of HIV PIC followed by viral DNA integration into the host cell genome are independent of both coreceptor usage and virus internalization mechanisms. Our results are in line with a recent report (65), but they are at variance with another showing a specific defect in the nuclear import of X4 virus PIC in macrophages (54), a discrepancy that could be due to the different methods used to detect nuclear HIV DNA rather than to the cell types: for example, the PCR detection of tandem 2 LTR circles as sole marker of nuclear HIV DNA is of low sensitivity (51, 66).

Because our data established that the block of HIV replication in mDC was exclusively due to postintegration mechanisms, we finally examined the capacity of mDC to ensure efficient HIV LTR transcription. Experiments performed with VSV-G-pseudotyped HIV-1 molecular clones expressing either GFP or Luciferase reporter genes revealed that HIV LTR transcription levels decreased by ~60–75% in DC upon maturation, the inhibition being stronger in DC CD40LT than in DC MCM. The mechanisms responsible for this phenomenon are still unclear. However, that mDC also displayed a reduced capacity to drive a CMV promoter suggests that this could result from interference with transcription initiation rather than with Tat-dependent transactivation and/or Rev-mediated splicing and cytoplasmic export of HIV RNAs. It is unlikely that such block in virus transcription is a general feature of gene regulation in mDC, inasmuch as whereas mDC are known to repress expression of receptors of inflammatory chemokines (CXCR1, CCR1, CCR2, CCR5, CCR6), CD1a, and molecules involved in Ag uptake, DC maturation is also associated with up-regulation of genes involved in T lymphocyte stimulation (adhesion and costimulatory molecules, CD54, CD58, and CD86; cytokines, IL-1,
IL-6, TNF, and IL-12) or recruitment (chemokines, MIP-1α/β and RANTES), and de novo expression of receptors (CXCR4 and CCR7) for tissue chemokines (67). In this study, in line with other reports (39, 68), we found that DC maturation, especially under the influence of CD40LT, led to the nuclear translocation of most NF-κB family members, which are known to activate HIV LTR transcription (55–58). However, inasmuch as it has been shown that mDC strongly up-regulate nuclear expression of c-Rel (68), which inhibits HIV transcription through competitive binding to the NF-κB sites of viral LTR with stimulatory NF-κB heterodimers (69), one cannot exclude its participation in the phenomenon described in this work. Alternatively, other negative regulatory factors, such as p53 or C/EBPα (70, 71), could also interfere with initiation of HIV transcription. Finally, although lack of SP1 expression has been suggested as a reason that DC cannot replicate HIV efficiently (39), neither SP1 nor SP3 transcription factors could be implicated in this study, since we found that DC maturation did not affect their high nuclear expression.

In conclusion, we have shown in this study that, in the same manner as macrophages and independently of their maturation stage, DC are less efficient at supporting reverse transcription of X4 than that of R5 HIV strains. More importantly, our data also indicate that the reduced capacity of mDC to support replication of R5 HIV is not related to a block of virus entry into cells, nor to decreased RE, nor to reduced viral DNA nuclear import or integration. We found that it is rather due to postintegration-negative regulatory events occurring mainly, if not exclusively, at the transcriptional level, which are apparently not related to alterations of the expression and nuclear localization of factors known to be involved in LTR transcription, such as NF-κB proteins and SP1 and SP3 transcription factors.

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