

# Activity of Reverse Transcriptase Inhibitors in Monocyte-Derived Dendritic Cells: A Possible *in Vitro* Model for Postexposure Prophylaxis of Sexual HIV Transmission

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## ABSTRACT

Because prevention of heterosexual HIV transmission is not always possible, it is important to develop effective strategies of postexposure prophylaxis (PEP). Since *in vivo* comparison of drug potency is difficult, we developed an *in vitro* model with cells resembling primary targets during sexual transmission: monocyte-derived dendritic cells (MO-DCs), Langerhans cells (MO-LCs), and resting autologous CD4<sup>+</sup> T cells. Nucleoside and nonnucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs, respectively) were evaluated for their antiviral activity, when added immediately after infection or at a later time point. In parallel, their immune-suppressive effect was examined by measuring inhibition of mixed MO-DC/allogeneic CD4<sup>+</sup> T cell cultures. Most RTIs potently inhibited HIV replication, even if added 24 hr after infection (representing PEP). The sensitivity to antiretroviral drugs was similar in HIV-infected MO-DCs and MO-LCs, but decreased in cocultures with resting autologous CD4<sup>+</sup> T cells. The NNRTIs efavirenz and UC-781 as well as the NRTIs AZT, 3TC, and d4T showed a similar high potency in MO-DC plus autologous CD4<sup>+</sup> T cell cocultures as compared with CEM T cells, whereas their activity in phytohemagglutinin/interleukin 2 (PHA/IL-2)-activated CD4<sup>+</sup> T cells was lower. The dideoxynucleoside RTI abacavir as well as the phosphonates (*R*)-PMPA and PMEA were more active in infected MO-DCs as compared with either CEM T cells or PHA/IL-2 activated CD4<sup>+</sup> T cells. Infection in cocultures of MO-DCs and autologous CD4<sup>+</sup> T cells could be aborted in a proportion of the cultures, with high concentrations of PMEA and/or efavirenz, but not with AZT. Suppressing activity in mixed leukocyte cultures was observed only at very high concentrations of RTI. Our data suggest that cocultures of MO-DCs and autologous CD4<sup>+</sup> T cells can be used as a possible *in vitro* model to explore protocols for PEP after sexual HIV transmission.

## INTRODUCTION

CONDOMS CAN EFFICIENTLY PREVENT sexual transmission of HIV and protective (vaginal) microbicides might become available in the near future. Nevertheless, prevention is not always possible, for example, during nonconsensual intercourse. Therefore, strategies of postexposure prophylaxis (PEP) must be explored. The presumption underlying PEP is that prompt antiretroviral treatment, probably in concert with HIV-specific

immune responses, may abort infection.<sup>1,2</sup> Antiretroviral PEP must act on primary target cells and by preference on the preintegration phase of the viral cycle. It must be applied within a short interval and at a sufficiently high concentration, without interfering with immune function.<sup>3</sup>

Reverse transcriptase inhibitors (RTIs), inhibiting a critical preintegration step of the viral cycle, are obvious candidates for PEP. Depending on their mode of action, RTIs are divided into nucleosides and nonnucleosides (NRTIs and NNRTIs, respec-

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tively). Among NRTIs, the 2′/3′-dideoxynucleoside analogs (e.g., zidovudine [AZT], lamivudine [3TC], stavudine [d4T], and didanosine [ddI]) require three phosphorylation steps for activation whereas the acyclic nucleotide phosphonate analogs (e.g., PMPA [9-(2-phosphonomethoxypropyl)adenine] and PMEA [9-(2-phosphonomethoxyethyl)adenine]) require only two phosphorylations, thus avoiding the first metabolic step, which is rate limiting for most dideoxynucleosides. The NNRTI compounds nevirapine, efavirenz, and UC-781 do not need metabolic activation and act at a site distinct from the enzymatic center.<sup>4</sup>

In humans, RTIs (including AZT and nevirapine) are efficient in preventing vertical transmission (preexposure prophylaxis)<sup>5–7</sup> and in lowering the risk of occupational, percutaneous transmission (postexposure prophylaxis).<sup>8</sup> These favorable results cannot automatically be extrapolated to sexual exposure because different primary target cells may be involved. PEP trials in humans after sexual HIV transmission are difficult, because large study groups are necessary and inclusion of placebo control subjects poses ethical problems. In some studies with macaques, various RTIs (including the NRTI PMPA and the NNRTI GW420867) aborted SIV, simian–human immunodeficiency virus (SHIV), or HIV-2 infection.<sup>9–12</sup> In most PEP experiments, animals were infected intravenously, except in the study by Otten *et al.*, who inoculated HIV-2 intravaginally.<sup>12</sup> In all these trials, PEP had to be initiated within 36 hr and maintained for 4 weeks to be fully effective. Clearly, studies of macaques are useful, but the number of available animals is also restricted. Therefore, *in vitro* modeling of PEP may offer a solution.

Combined evidence from *in vivo* and *in vitro* studies suggests that dendritic cells (DCs), either intraepithelial Langerhans cells (LCs) or subepithelial (interstitial) DCs, are early targets for HIV after sexual transmission.<sup>13,14</sup> Virus-bearing DCs migrate to secondary lymphoid organs and interact with CD4<sup>+</sup> T cells, resulting in HIV-specific immune responses, but also in productive infection of CD4<sup>+</sup> T cells.<sup>15–17</sup> Importantly, during sexual transmission, non-syncytium-inducing and coreceptor CCR5–using (NSI/R5) viral clones are selected over syncytium-inducing and CXCR4–using (SI/X4) clones.<sup>18,19</sup> Therefore, the classically used T cell lines, selectively permissive to SI/X4 clones, are not appropriate to mimic PEP *in vitro*. Infecting DCs or LCs with HIV and culturing them with resting autologous CD4<sup>+</sup> T cells seems more relevant for PEP. Because the isolation of primary *ex vivo* DCs is not feasible for large studies, *in vitro* models have been proposed. The interstitial DC type can be generated from monocytes (MOs), using granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin 4 (IL-4).<sup>20,21</sup> Culturing MOs with GM-CSF, IL-4, and transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) results in an LC-type cell.<sup>22</sup> These two models will be referred to as MO-DCs and MO-LCs, respectively.

In the present article, we study the effects of various RTIs of the three subclasses in MO-DCs (and MO-LCs), in cocultures with resting autologous CD4<sup>+</sup> T cells, and compare their antiviral effect in phytohemagglutinin (PHA)/IL-2-activated CD4<sup>+</sup> T cells and in CEM T cells as a reference. The “window of opportunity” for viral suppression is defined in time-lapse experiments, the “immune-suppressive” effect of RTIs is studied in mixed leukocyte cultures (MLCs), and conditions for abortion of infection in DC cultures are explored.

## MATERIALS AND METHODS

### *Immune phenotyping*

The following antibodies were used: anti-CD1a conjugated with fluorescein isothiocyanate (anti-CD1a-FITC), anti-CD40-FITC (Biosource Europe, Nivelles, Belgium), anti-CD3-FITC, anti-CD4-phycoerythrin (PE), anti-CD13-PE, anti-CD14-FITC, anti-CD80-PE, anti-IgG1-FITC, and anti-DC-SIGN-PE (Becton Dickinson, Erembodegem, Belgium). One hundred thousand cells in 50  $\mu$ l of medium were incubated with 3 to 5  $\mu$ l of antibodies (30 min at 4°C). Cells were washed, fixed with 1% paraformaldehyde, and analyzed on a FACScan (BD Immunocytometry Systems, San Jose, CA).

### *Generation of monocyte-derived interstitial-type dendritic cells, monocyte-derived Langerhans-type cells, and CD4<sup>+</sup> T cells*

Dendritic cell generation was based on the protocols of Salusto and Lanzavecchia,<sup>20</sup> Romani *et al.*,<sup>21</sup> and Geissmann *et al.*,<sup>22</sup> with minor modifications as described.<sup>23</sup> Briefly,  $6 \times 10^8$  peripheral blood mononuclear cells (PBMCs), isolated from donor buffy coats (kindly provided by the Antwerp Red Cross Blood Transfusion Center), were separated into monocyte- and lymphocyte-enriched fractions by counterflow elutriation. The monocytes (MOs) were further purified by sheep erythrocyte rosetting, yielding >90% CD3<sup>-</sup>CD4<sup>+</sup> MOs and <0.5% T cells. MOs were cultured for 6–7 days in RPMI 1640 (BioWhittaker, Verviers, Belgium), supplemented with 10% bovine calf serum (BCS) (HyClone, Logan, UT), penicillin (100 U/ml), and streptomycin (100  $\mu$ g/ml) (Boehringer-Mannheim, Mannheim, Germany), further referred to as complete medium. To differentiate MOs into interstitial-type dendritic cells (MO-DCs), GM-CSF (20 ng/ml) and IL-4 (20 ng/ml) (Immunosource, Zoersel, Belgium) were added. Half the medium, containing the same concentration of cytokines, was replaced after 3–4 days. For differentiation of MOs into Langerhans-type cells (MO-LCs), the complete medium was supplemented with GM-CSF (20 ng/ml), IL-4 (20 ng/ml), and natural TGF- $\beta$ 1 (5 ng/ml) (Immunosource).

The lymphocyte fraction was frozen in liquid nitrogen and thawed on the day of infection. CD4<sup>+</sup> T cells were purified by positive selection, using a CD4<sup>+</sup> isolation kit (Dynal, Oslo, Norway), as described.<sup>23,24</sup> They were >95% CD4<sup>+</sup> and CD3<sup>+</sup>.

### *HIV strains and HIV antigen detection*

In most experiments with MO-DCs, the NSI/R5, monotropic strain Ba-L, kindly provided by the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program (Rockville, MD), was used. We did one experiment with CI22 and VII363, two NSI/R5 subtype B isolates from our own collection. The SI/X4, lymphotropic laboratory strain HTLV-III<sub>B</sub> was originally obtained from R.C. Gallo and M. Popovic (at that time at the NIH, Bethesda, MD). To determine the infectious titer of the viral stocks, donor PBMCs were preactivated with PHA (0.5  $\mu$ g/ml; Innogenetics, Ghent, Belgium) and IL-2 (5 ng/ml; Immunosource) for 3 days. They were seeded in 96-well plates at  $7.5 \times 10^4$  cells per well and infected 6-fold with a serial one-third dilution of the viral stocks. After 7 days of culture with IL-2, titers were determined as the TCID<sub>50</sub> (50%

tissue culture infective dose), according to the method of Reed and Muench<sup>25</sup> as modified by Peden and Martin.<sup>26</sup>

To measure the level of HIV antigen in the supernatants, both for determination of TCID<sub>50</sub> and of the 50% effective drug concentration (EC<sub>50</sub>; see below), we used an in-house enzyme-linked immunosorbent assay (ELISA), the characteristics of which have been described.<sup>27</sup> Briefly, this assay uses purified IgG from HIV-seropositive subjects, selected for a broad recognition of all HIV-1 and HIV-2 subtypes. Although p24 is the dominant antigen, other HIV proteins are recognized by these polyclonal antibodies. The lower detection limit is about 200 pg/ml and the upper limit is about 25,000 pg/ml. Optical densities (ODs) are transformed into HIV antigen concentrations, using a standard curve of Ba-L stock dilutions, the p24 content of which was preliminarily determined with a commercial kit (Innogenetics).

#### *Measuring 50% effective concentration of antiretroviral drugs*

In the group of dideoxynucleoside NRTIs, zidovudine (AZT), lamivudine (3TC), didanosine (ddI), stavudine (d4T), and abacavir (ABC) were tested. The acyclic nucleoside phosphonate NRTI adefovir (PMEA) and tenofovir (PMPA) were used. Efavirenz and UC-781 (a thiocarboxanilide derivative) were selected as representative potent NNRTIs. All stocks were made in dimethyl sulfoxide (DMSO) at 4 to 25 mM.

MO-DCs and MO-LCs (from the same donor) were suspended at  $10 \times 10^6$  cells/ml in complete medium and incubated for 2 hr at 37°C with Ba-L at a multiplicity of infection (MOI) of  $10^{-3}$ . Afterward, cells were washed at least six times and resuspended at  $2 \times 10^6$  cells/ml in complete medium. Fifty microliters of DCs was dispensed in a 96-well plate and 50  $\mu$ l of either medium or resting autologous CD4<sup>+</sup> T cells (at  $2 \times 10^6$ /ml) was added. Drugs (100  $\mu$ l), prepared in a 5-fold dilution series in complete medium, were added immediately after infection or 24 hr later (the latter representing PEP). Each drug concentration was tested in 2-fold in the preliminary experiments (Table 1 and Fig. 2) and in 6-fold in all subsequent experiments. Half the culture medium, including the drugs (but without any added cytokine), was replaced twice every week. After 2 weeks, HIV antigen was measured in 100  $\mu$ l of Nonidet P-40 (NP-40)-inactivated culture supernatant, using our in-house ELISA assay. HIV antigen concentration was plotted against drug concentration and regression analysis was done on the linear part of the curve to calculate the EC<sub>50</sub> value (equivalent to the drug concentration required to reduce HIV antigen production by 50%).

As a first reference system, CD4<sup>+</sup> T cells were purified from buffy coat, using Dynabeads, and preactivated for 3 days with PHA (0.5  $\mu$ g/ml) and IL-2 (5 ng/ml) (further referred to as PHA/IL-2-activated CD4<sup>+</sup> T cells). Infection with Ba-L and drug treatment proceeded as described for MO-DCs. The cells were cultured at a concentration of  $0.5 \times 10^6$ /ml in IL-2-containing medium. Half the medium, containing drugs and IL-2, was replaced twice a week. The resulting HIV antigen concentration was measured after 2 weeks to calculate the EC<sub>50</sub>.

As a second reference system, CEM T cells (obtained from the American Type Culture Collection, Rockville, MD) were used under previously standardized conditions.<sup>28</sup> Briefly, cells were suspended at 250,000 cells/ml in RPMI 1640, sup-

plemented with 10% fetal calf serum, 2 mM L-glutamine, and 0.075% NaHCO<sub>3</sub> and infected with HTLV-III<sub>B</sub> at  $\sim 20$  TCID<sub>50</sub>. In one set of experiments, 100- $\mu$ l aliquots of a 5-fold dilution series of the drugs were immediately added to 100- $\mu$ l samples of the infected cells in 200- $\mu$ l well plates. In a second set of experiments, the compounds were added 24 hr after infection. After 4 to 5 days of incubation at 37°C, the cultures were examined for syncytium formation. The EC<sub>50</sub> is the concentration required to inhibit syncytium formation by 50%.

#### *Determining the therapeutic window*

Ba-L-infected MO-DCs were cocultured with resting autologous CD4<sup>+</sup> T cells. AZT and/or efavirenz were added at various concentrations after 24, 48, or 72 hr and maintained in culture as described. Two weeks later HIV antigen was measured.

#### *Testing for abortion of infection by adding PHA/IL-2-activated PBMCs and measuring proviral DNA*

The primary culture was set up as usual with Ba-L-infected MO-DCs and resting, autologous CD4<sup>+</sup> T cells. Anti-HIV drugs were added 24 hr after infection. After 2 weeks, 100  $\mu$ l of supernatant was taken for HIV antigen detection in our ELISA. The cells were washed three times to remove the drugs and a secondary culture was set up by adding 200  $\mu$ l of PHA/IL-2-activated PBMCs ( $0.5 \times 10^6$  cells/ml in complete medium, supplemented with IL-2 at 5 ng/ml). Half the culture medium was replaced every 3–4 days with IL-2-containing medium and supernatants as well as cells were harvested after an additional 3 weeks. The supernatants were tested for HIV antigen. The cells, consisting of >90% T cells, were processed for HIV DNA measurement, using a polymerase chain reaction (PCR)-based HIV proviral DNA quantitation kit developed from the Amplicor HIV-1 Monitor test, version 1.5 (Roche Molecular Systems, Branchburg, NJ), the modifications of which have been described.<sup>29</sup> A lower threshold of 10 HIV copies per  $10^6$  cells was confirmed by using 8E5/LAV cells, containing 1 copy of proviral DNA per cell (kindly provided by the Centralized Facility for AIDS Reagents of NIBSC, Potters Bar, UK).

#### *Immune-suppressive activity of antiretroviral drugs*

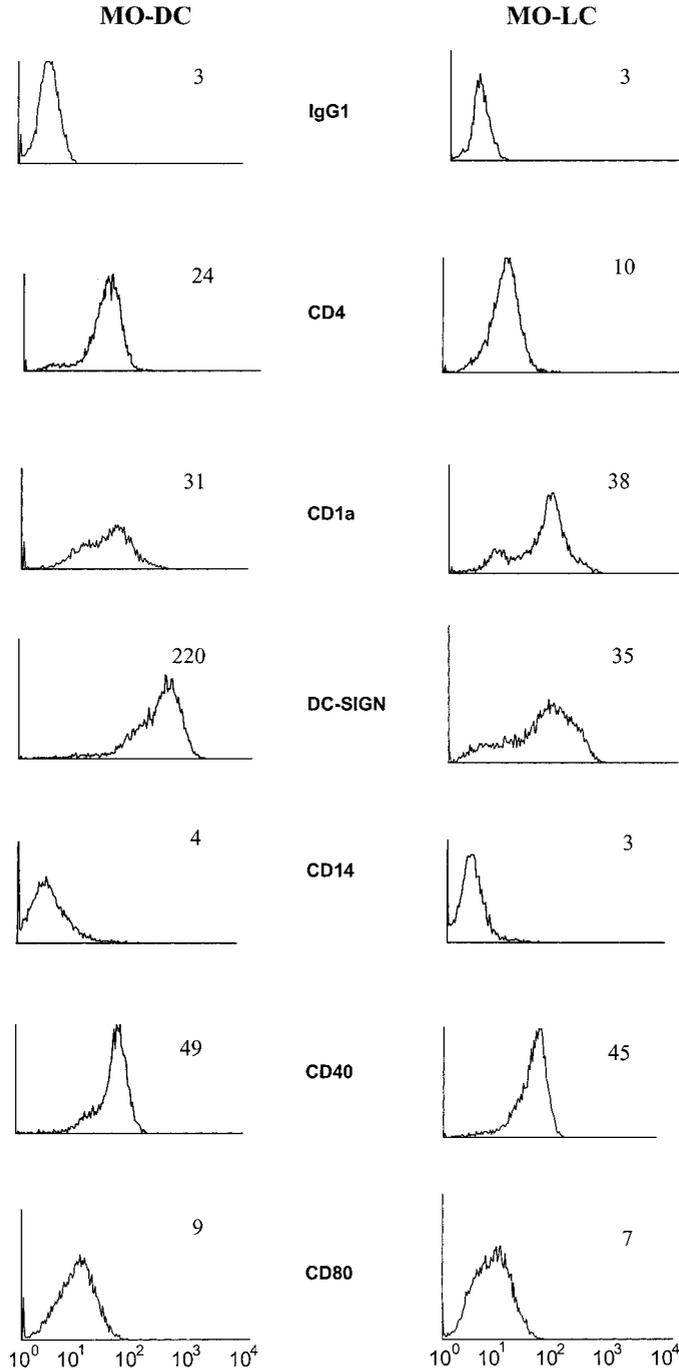
Immune-suppressive activity of the drugs was determined in a mixed leukocyte culture (MLC), using MO-DCs as stimulators and allogeneic (resting) CD4<sup>+</sup> T cells as responders in RPMI 1640, supplemented with antibiotics and 2.5% pooled human serum (obtained from healthy blood donors). Quadruplicate cultures of  $3 \times 10^3$  MO-DCs and  $100 \times 10^3$  allogeneic CD4<sup>+</sup> T cells were set up in a 96-well plate, in the presence or absence of one or two anti-HIV drugs at various concentrations. After 5 days, 1  $\mu$ Ci of [*methyl*-<sup>3</sup>H]thymidine (TRA.120; Amersham Pharmacia, Buckinghamshire, UK) was added to each well. Plates were harvested 7 hr later, and [*methyl*-<sup>3</sup>H]thymidine incorporation was measured in a scintillation counter (Top Count; Canberra-Packard, Zellik, Belgium) and expressed as counts per minute (cpm). The immune-suppressive concentration (ISC<sub>50</sub>) is defined as the drug concentration inhibiting 50% of the lymphocyte proliferation.

**RESULTS**

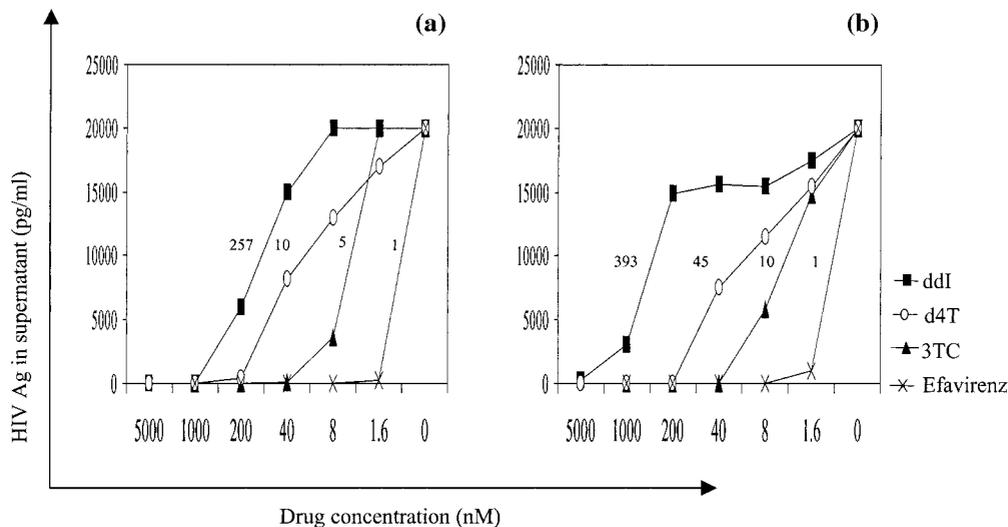
*In vitro-differentiated MO-DCs and MO-LCs resemble their in vivo counterparts*

MO-DCs and MO-LCs, *in vitro* differentiated out of monocytes in the presence of GM-CSF plus IL-4 (MO-DCs) or

GM-CSF plus IL-4 plus TGF- $\beta$ 1 (MO-LCs), were phenotypically analyzed (Fig. 1). Both MO-DCs and MO-LCs expressed CD4 and costimulatory molecules. Moreover, DC-SIGN expression was 6-fold higher on MO-DCs, compared with MO-LCs. CD14 expression was low to absent in both cell types. The expression of CD40 and CD1a was similar for both MO-DCs and MO-LCs. Both MO-DCs and MO-LCs



**FIG. 1.** Phenotypical characteristics of MO-derived interstitial type and Langerhans-type dendritic cells (MO-DCs and MO-LCs, respectively). MO-DCs and MO-LCs, derived from the same donor, were analyzed with anti-CD4, anti-CD1a, anti-CD40, anti-CD14, anti-DC-SIGN, and anti-CD80 monoclonal antibodies. Anti-IgG1 was included as an isotypic control. Fluorescence intensity is shown as the geometric log mean ( $G_{mean}$ ) value for each marker.



**FIG. 2.** Antiviral activity of reverse transcriptase inhibitors toward Ba-L replication in MO-DCs and MO-LCs. MO-DCs and MO-LCs, derived from the same donor, were infected with  $10^{-3}$  MOI of Ba-L. A dilution series of each RTI was added to duplicate cultures immediately after infection and maintained at the same concentration for 2 weeks. HIV antigen (Ag) was measured in the supernatants by ELISA and is represented as picograms per milliliter for cultures of MO-DCs (a) and MO-LCs (b). Fifty percent effective drug concentrations ( $EC_{50}$ ), calculated in nanomolar ( $\mu M$ ) by linear regression analysis, are indicated.

vigorously stimulated allogeneic resting T cells (data not shown).

#### *RTIs have similar anti-HIV activity in MO-DCs and MO-LCs*

Ba-L-infected MO-DCs and MO-LCs from the same donor, cultured either alone or with noninfected resting autologous  $CD4^+$  T cells, were compared for their sensitivity to the antiviral activity of various RTIs. In a first set of experiments, three representative NRTIs (3TC, d4T, and ddi) and one NNRTI

(efavirenz) were used. The order of drug potency, as reflected by  $EC_{50}$  values, was the same: efavirenz > 3TC > d4T > ddi (compare Fig. 2a with 2b). In cocultures with resting autologous  $CD4^+$  T cells, virus production increased and the  $EC_{50}$  of 3TC, d4T, and ddi (but not efavirenz) shifted to higher values. Overall,  $EC_{50}$  values were similar in cultures containing MO-DCs or MO-LCs and the inhibitory activity was maintained, irrespective of immediate or 24-hr-delayed drug addition (Table 1). Comparison of PMPA, PMEA, and abacavir in a second set of experiments confirmed a similar sensitivity of MO-DCs and MO-LCs to these NRTIs, as well (data not

TABLE 1. ANTIVIRAL ACTIVITY OF REVERSE TRANSCRIPTASE INHIBITORS IN MONOCYTE-DERIVED INTERSTITIAL TYPE DENDRITIC CELLS AND LANGERHANS-LIKE CELLS

Drug	Time (hr)	$EC_{50}$ (nM) $CD4^+$			
		MO-DCs <sup>a</sup>	MO-DCs + $CD4^+$ T cells <sup>a</sup>	MO-LCs <sup>a</sup>	MO-LCs + $CD4^+$ T cells <sup>a</sup>
<b>NRTI</b>					
3TC	0 <sup>b</sup>	5 <sup>c</sup>	25	10	24
	24 <sup>b</sup>	16	90	13	ND <sup>d</sup>
d4T	0	10	142	45	445
	24	73	382	77	254
ddi	0	257	4400	393	1373
	24	188	>5000	351	>5000
<b>NNRTI</b>					
Efavirenz	0	1	1	1	2
	24	3	4	2	12

<sup>a</sup>MO-DCs and MO-LCs from the same donor were infected with Ba-L and cultured alone or with autologous  $CD4^+$  T cells.

<sup>b</sup>Drugs were added immediately or 24 hr after infection and maintained for 2 weeks of culture.

<sup>c</sup>Fifty percent effective drug concentrations ( $EC_{50}$ , nanomolar) were calculated by linear regression analysis.

<sup>d</sup>ND, not determined.

TABLE 2. ANTIVIRAL AND IMMUNE-SUPPRESSIVE ACTIVITY OF SELECTED RTIs IN DIFFERENT TARGET CELLS<sup>a</sup>

Drug	Time (hr)	EC <sub>50</sub> (nM) <sup>b</sup>					TI <sup>g</sup>
		CEM T cells <sup>c</sup>	PHA/IL-2 CD4 <sup>+</sup> T cells <sup>d</sup>	MO-DCs <sup>e</sup>	MO-DCs + CD4 <sup>+</sup> T cells <sup>e</sup>	ISC <sub>50</sub> (nM) <sup>f</sup>	
AZT	0	9 (6–10)	554 (212–1,450)	19 (3–70)	70 (25–671)	5,015 (2,862–10,294)	72
	24	6 (4–10)	6,624 (5838–7515)	25 (2–77)	20 (3–98)		
3TC	0	88 (61–140)	241 (235–247)	14 (3–118)	67 (17–415)	>25,000	>373
	24	57 (44–79)	262 (230–298)	33 (11–406)	138 (27–496)		
d4T	0	288 (200–400)	1,736 (1,602–1,881)	71 (10–480)	379 (123–2,219)	17,535 (13,325–25,215)	46
	24	262 (200–300)	4,691 (5,754–3,824)	160 (23–650)	349 (23–2,931)		
ABC	0	5,429 (4,000–10,000)	2,005 (2,118–1,899)	27 (12–54)	377 (46–3,575)	16,204 (12,931–20,866)	43
	24	6,694 (5,000–10,000)	2,603 (3,291–2,059)	63 (35–84)	782 (331–2,746)		
ddl	0	8,434 (6,000–10,000)	>7,477 (5,591–>10,000)	207 (73–470)	3,858 (3,382–4,400)	>25,000	>7
	24	4,472 (4,000–5,000)	>10,000	558 (188–1,655)	>5000		
(R)-PMPA	0	4,759 (2,200–7,000)	4,345 (3,213–5,876)	118 (68–673)	460 (96–2,593)	>25,000	>54
	24	1,776 (800–2,800)	3,472 (5,500–2,192)	441 (48–2,193)	962 (528–2,940)		
PMEA	0	8,320 (6,000–12,000)	4,462 (6,298–3,161)	194 (60–2,305)	479 (89–2,430)	21,976 (18,703–28,897)	46
	24	4,379 (2,000–6,000)	6,937 (8,361–5,755)	595 (203–2,876)	1,902 (711–3,258)		
Efavirenz	0	3 (2–4)	32 (47–22)	2 (1–15)	4 (1–24)	16,077 (13,916–18,452)	4,019
	24	3 (2–4)	43 (54–35)	3 (2–3)	9 (4–27)		
UC-781	0	8 (7–9)	42 (27–65)	7 (2–25)	36 (22–130)	11,512 (4,645–14,930)	320
	24	7 (6–9)	91 (550–15)	8 (2–90)	65 (21–424)		

<sup>a</sup>Values are given as geometric means (minimum–maximum) of at least four independent experiments, except for the EC<sub>50</sub> of PHA/IL-2-stimulated CD4<sup>+</sup> T cells: geometric mean of two independent experiments, with individual values in parentheses.

<sup>b</sup>EC<sub>50</sub> (nM), 50% effective concentration.

<sup>c</sup>CEM T cells were infected with HTLV-III<sub>B</sub>.

<sup>d</sup>Purified CD4<sup>+</sup> T cells were preactivated with PHA/IL-2, infected with Ba-L, and maintained in IL-2 medium.

<sup>e</sup>MO-DCs were infected with Ba-L and cultured alone or with resting autologous CD4<sup>+</sup> T cells, without cytokines.

<sup>f</sup>ISC<sub>50</sub> (nM), 50% immune-suppressive concentration, as measured in allogeneic MO-DC/CD4<sup>+</sup> T cell cocultures (see Fig. 4).

<sup>g</sup>TI, Therapeutic index: ISC<sub>50</sub>/EC<sub>50</sub> of MO-DCs + CD4<sup>+</sup> T cells.

shown). We decided to focus on MO-DCs for more extensive experiments.

*RTIs are potent inhibitors of HIV replication in MO-DCs alone or in coculture with resting autologous CD4<sup>+</sup> T cells, even if added 24 hr postinfection*

The antiviral activity of RTIs was evaluated in Ba-L-infected MO-DCs and in cocultures of MO-DCs plus resting autologous CD4<sup>+</sup> T cells and compared with the activity in Ba-L-infected PHA/IL-2-activated CD4<sup>+</sup> T cells and HTLV-III<sub>B</sub>-infected CEM T cells. Drugs were added immediately after infection and 24 hr later in parallel (Table 2).

In the classic test system of HTLV-III<sub>B</sub>-infected CEM T cells, the NNRTIs efavirenz and UC-781, as well as the NRTIs AZT, 3TC, and d4T, were active in the submicromolar range, whereas ABC, ddI, and the phosphonates were less potent. In cultures of Ba-L-infected PHA/IL-2-activated CD4<sup>+</sup> T cells, the latter products showed EC<sub>50</sub> values similar to those in CEM T cells, whereas the EC<sub>50</sub> values of the former, more potent drugs were higher (approximately 0.5 log for 3TC, 1 log for d4T and efavirenz and UC-781, and 2–3 logs for AZT).

In cultures of Ba-L-infected MO-DCs (either alone or with resting autologous CD4<sup>+</sup> T cells) all drugs were clearly more active (EC<sub>50</sub> 1–2 logs lower) as compared with PHA/IL-2 activated CD4<sup>+</sup> T cells. The EC<sub>50</sub> was systematically higher in cocultures of MO-DCs and resting autologous CD4<sup>+</sup> T cells, as compared with MO-DCs alone.

In all cell systems the antiviral activity was maintained if addition of the drugs was postponed for 24 hr. Obviously, for PEP testing, coculture of infected MO-DCs plus resting autologous CD4<sup>+</sup> T cells and addition of drugs after 24 hr is most relevant. In this setting, efavirenz and UC-781, as well as AZT and

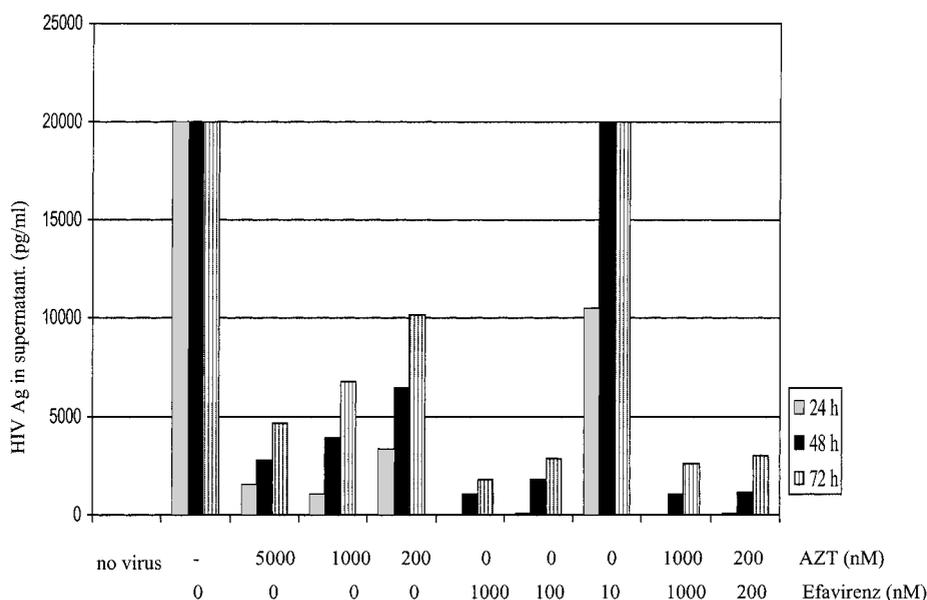
3TC, were very potent (submicromolar range), whereas most other compounds were active at about 1 μM. The NRTI ddI was the weakest drug.

Comparing PEP of AZT and efavirenz after 24, 48, and 72 hr in cocultures of MO-DCs plus resting autologous CD4<sup>+</sup> T cells showed that the antiviral effect was most efficient after 24 hr. Inhibition of viral replication became much more difficult when drug was added at a later time (Fig. 3).

*Viral rescue in infected MO-DCs can be prevented in a proportion of cultures by RTIs*

High concentrations of RTI apparently block HIV replication in cocultures of MO-DCs plus resting autologous CD4<sup>+</sup> T cells, as measured by ELISA. In view of the low sensitivity of ELISA, a low-grade or latent infection might go undetected. To prove or disprove abortion of infection, we decided to set up "rescue experiments," in which the PEP treatment was stopped after 2 weeks (by thoroughly washing the cells free of drugs) and sensitive target cells (PHA/IL-2-activated PBMCs) were added to amplify a low-grade infection. Because NNRTIs and NRTIs have a different submolecular target and because 2'3'-dideoxynucleoside NRTIs and acyclic nucleotide phosphonate NRTIs have a distinct metabolism, we decided to test one representative of each RTI subclass (efavirenz, AZT, and PMEA, respectively), either alone or in combination in three independent sets of experiments.

In the first experiment, efavirenz at 200 or 1000 nM and PMEA at 5000 nM completely suppressed HIV production even after addition of PHA/IL-2-activated PBMCs: HIV could not be found as antigen in the supernatant, or as proviral DNA in the cells. Moreover, whereas efavirenz at 50 nM and PMEA at 1000 nM separately did not prevent viral rescue, combined



**FIG. 3.** Influence of delayed RTI addition on suppression of HIV replication. MO-DCs were infected with  $10^{-3}$  MOI of Ba-L (except in the "no virus" condition), washed extensively, and cultured in the presence of autologous CD4<sup>+</sup> T cells. AZT, efavirenz, or a combination of both was added 24, 48, or 72 hr after infection and maintained at the indicated concentrations. After 13 days of culture, HIV antigen was measured by ELISA.

TABLE 3. EVALUATION OF VIRAL RESCUE BY PHA/IL-2-ACTIVATED PBMCs IN DRUG-TREATED COCULTURES OF MO-DCs PLUS RESTING AUTOLOGOUS CD4<sup>+</sup> T CELLS<sup>a</sup>

Concentration (nM)		HIV antigen (number of positive wells) <sup>b</sup>				
		Exp. 1 <sup>c</sup>	Exp. 2		Exp. 3	
Efavirenz	PMEA	(2° culture)	1° culture	2° culture	1° culture	2° culture
0	0	6/6	6/6	6/6	6/6	6/6
0	1000	6	6	6	6	6
0	5000	0 <sup>e</sup>	6	6	6	6
10	0	ND <sup>d</sup>	6	6	6	6
50	0	6	1	5	0	5
200	0	0	0	4	0	4
1000	0	0	0	2	0	2
10	1000	ND <sup>d</sup>	<b>4</b>	<b>5</b>	6	6
50	1000	<b>0</b>	0	<b>3</b>	0	<b>4</b>
200	1000	0	0	<b>3</b>	0	<b>1</b>
1000	1000	ND <sup>d</sup>	0	2	0	<b>0</b>

<sup>a</sup>MO-DCs (from donors A, B, and C in experiments 1, 2, and 3) were infected with 10<sup>-3</sup> MOI of Ba-L and cocultured with autologous CD4<sup>+</sup> T<sup>+</sup> cells in 6-fold microcultures. After 24 hr, drugs were added at the indicated concentrations and maintained for 2 weeks of primary (1°) culture. Afterward, cells were washed and PHA/IL-2-activated PBMCs were added and maintained in IL-2-containing medium during a secondary (2°) culture of 3 weeks.

<sup>b</sup>Each supernatant was tested for HIV antigen by ELISA. The number of antigen-positive microcultures is represented. Possible additive effects are printed in boldface.

<sup>c</sup>Note that in experiment 1 only the results of the 2° culture are available.

<sup>d</sup>ND, Not done (the lowest concentration of efavirenz was not used in experiment 1).

<sup>e</sup>In each case, when the ELISA remained negative in all 6 microcultures at the end of the 2° culture, proviral HIV DNA was below the detection limit in the PHA/IL-2-activated PBMCs, whereas it was clearly positive in all other cases (data not shown).

treatment apparently aborted infection in all cultures (Table 3, Exp. 1).

In the second and third experiment complete abortion of HIV was more difficult to obtain. First, PMEA alone at 5000 nM failed to block HIV replication, even during the primary (1°) culture. Conversely, efavirenz strongly suppressed HIV replication at 50 nM during the 1° culture, as expected, but the virus was rescued after addition of PHA/IL-2-activated PBMCs in five of six wells of the secondary (2°) cultures. At higher efavirenz concentrations, the 1° cultures remained negative, but the virus was still rescued in four and two wells at 200 and 1000 nM, respectively. Combining both drugs provided some evidence of additive effects, in that virus was rescued in a lower proportion of the wells, as compared with pretreatment with efavirenz alone. However, complete abortion was seen only in experiment 3, with the combination of efavirenz at 1000 nM and PMEA at 1000 nM (Table 3) or 5000 nM (not shown).

To our surprise, AZT consistently failed to abort the infection, even at 5000 nM, and it had no clear-cut additive effect in combination with efavirenz (data not shown).

#### Primary isolates are at least as sensitive to inhibition by the NNRTI efavirenz as HIV Ba-L

To investigate whether results obtained with Ba-L also apply to primary isolates, MO-DCs from one donor were infected in parallel with Ba-L and two NSI/R5 subtype B primary iso-

lates at the same multiplicity of infection and cultured in the presence of resting autologous CD4<sup>+</sup> T cells. An efavirenz-based PEP treatment was initiated 24 hr postinfection. At the end of primary culture, all three HIV strains seemed approximately equally sensitive to inhibition by efavirenz, because 10 nM efavirenz was sufficient to inhibit viral replication of all microcultures. After secondary culture with PHA/IL-2-activated PBMCs (in the absence of drug), a pretreatment of 1000 nM efavirenz was needed to completely prevent rescue of Ba-L, whereas 100 and 10 nM efavirenz sufficed to abort infection of CI22 and VII363 respectively (Table 4).

#### RTIs have low immune-suppressive activity

The interaction between HIV-infected MO-DCs and CD4<sup>+</sup> T cells is responsible for initiation of anti-HIV immune responses. Because functional impairment might occur at drug concentrations well below cell death-inducing doses, we evaluated the inhibitory effect of the drugs on proliferative T cell responses to allogeneic MO-DCs. Individual drugs were tested in three experiments: a representative experiment is shown in Fig. 4. Only the highest concentrations of most drugs had a negative effect on the MLC, implying 50% immune-suppressive concentrations (ISC<sub>50</sub>) in the 5000–25,000 nM range or above. As a consequence, therapeutic indices (TIs) were high for all drugs tested (Table 2). Combinations of efavirenz with AZT, 3TC, or PMPA, at concentrations up to 5000 nM, failed to inhibit MLC responses (data not shown).

TABLE 4. SENSITIVITY TO INHIBITION BY NONNUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR EFAVIRENZ: COMPARISON OF PRIMARY ISOLATES AND HIV Ba-L

HIV strain <sup>a</sup>	Efavirenz (nM) <sup>a</sup>	HIV antigen (number of positive wells)	
		1° culture <sup>b</sup>	2° culture <sup>b</sup>
Ba-L	1000	0/6	0/6
	100	0	1
	10	0	5
	1	5	6
CI22	1000	0	0
	100	0	0
	10	0	1
	1	2	4
VI1363	1000	0	0
	100	0	0
	10	0	0
	1	2	6

<sup>a</sup>MO-DCs were infected with  $10^{-3}$  MOI of the indicated HIV strains and PEP-treated with efavirenz.

<sup>b</sup>Primary and secondary cultures were performed as in the experiments of Table 3. HIV antigen in the supernatants of primary and secondary cultures was measured by ELISA. The number of antigen-positive microcultures is represented. At the end of primary culture, HIV antigen values were measured in untreated controls and found to be 9330, 1716, and 2123 pg/ml for, respectively, Ba-L-, CI22-, and VI1363-infected cells.

## DISCUSSION

Dendritic cells were found to be important target cells for sexual HIV transmission.<sup>14,17</sup> We developed an *in vitro* model based on MO-DCs/MO-LCs and autologous CD4<sup>+</sup> T cells to explore protocols for postexposure prophylaxis after sexual HIV transmission. In our model all types of RTI inhibited HIV infection at doses far below immune-suppressive concentrations and the inhibition was sustained if drug treatment was postponed for 24 hr. A 2-week treatment with high doses of the NNRTI efavirenz apparently blocked HIV replication, but viral rescue was occasionally observed after addition of sensitive PHA/IL-2-activated PBMCs. Coadministration of the acyclic nucleotide phosphonate PMEA, but not of the 2'3'-dideoxynucleoside AZT, showed some additive effects.

It remains controversial whether the first targets of HIV in the rectum, uterus, and cervix are subepithelial immature interstitial DCs or intraepithelial LCs.<sup>14,17,30</sup> These *in vivo* target cells can be modeled by MO-DCs and MO-LCs, respectively. Both cell types potently activated resting T cells, were susceptible to infection with NSI/R5 viruses, and expressed CD4 and costimulatory molecules whereas the monocyte-macrophage marker CD14 was strongly downregulated.<sup>31</sup> However, both MO-DCs and MO-LCs expressed CD1a, a marker that is absent on interstitial DCs but present on subepithelial Langerhans cells *in vivo*. The DC-SIGN molecule, important for *trans*-infection of CD4<sup>+</sup> T cells, was expressed on MO-DCs and, to a lesser extent, on MO-LCs, whereas *in vivo*, DC-SIGN expression is found only on interstitial DCs and not on primary LCs.<sup>32,33</sup> Clearly, MO-DCs and MO-LCs display an intermediate phenotype. Moreover, our first experiments pointed to a

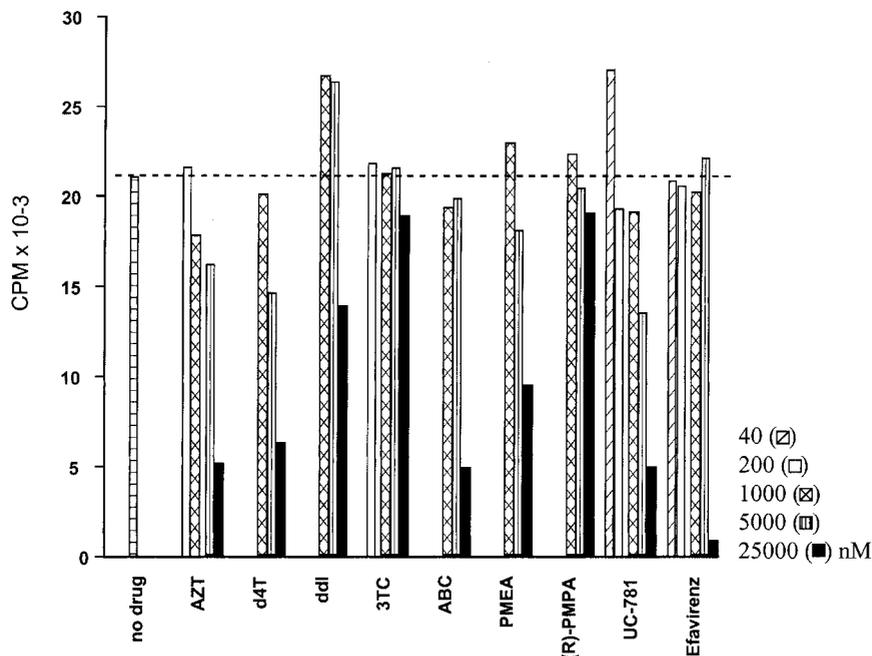


FIG. 4. Immune suppression by RTIs. A mixed leukocyte culture was set up with MO-DCs as stimulators and allogeneic CD4<sup>+</sup> T cells as responders, in the presence of RTIs at the indicated concentrations. [*methyl*-<sup>3</sup>H]Thymidine incorporation after 5 days is expressed as counts per minute (cpm).

similar antiviral activity of RTIs in MO-DCs and MO-LCs. We decided to focus on the model of MO-DCs.

A second point of debate is whether HIV actually infects DCs or only attaches to the surface.<sup>34-37</sup> We previously clearly showed that the prototypical NSI/R5 Ba-L, as well as various primary strains, productively infected MO-DCs and were efficiently transferred to either resting or activated T cells.<sup>23,24,31</sup>

Because our model is static in nature, we could not mimic transport of cells from mucosa to lymph nodes, during which dynamic interactions occur between DCs, T cells, and other cells of the innate and adaptive immune system. However, even in whole organ cultures, the dynamics of cell trafficking are not fully addressed.<sup>38</sup> A recent report showed that after acute HIV infection, DCs from the lymph nodes express Cd1a, DC-SIGN, and co-stimulatory molecules after acute HIV infection,<sup>39</sup> which are similar to the cells we generated *in vitro*. A mucosal barrier was not modeled in our system because this is, to our knowledge, of minor importance in the exploration of postexposure prophylaxis. In fact, by definition, PEP begins after virus has already passed the mucosa and has infected the first subepithelial cells.

Our data indicated that Ba-L-infected MO-DCs alone were equally or more sensitive to RTIs, as compared with HTLVIII<sub>B</sub>-infected CEM T cells, and clearly more sensitive than Ba-L-infected PHA/IL-2-activated CD4<sup>+</sup> T cells. This seems surprising, because the cellular kinases, required for NRTI phosphorylation, are not active in nonproliferating cells, including DCs. On the other hand, noncycling cells have lower endogenous levels of dideoxynucleoside triphosphates, which compete with activated NRTIs for binding to the enzymatic site of HIV RT.<sup>40,41</sup> Adding resting autologous CD4<sup>+</sup> T cells to infected MO-DCs increased the EC<sub>50</sub> values of most NRTIs. Previously, we demonstrated that MO-DCs actually activate autologous T cells,<sup>24</sup> thus presumably favoring nucleoside phosphorylation and increasing NRTI activity. However, T cell activation also stimulates viral replication and augments the endogenous dideoxynucleoside triphosphates pools, counteracting NRTI activity.<sup>41</sup> The latter effects of T cell activation may also explain the clearly higher doses of antiviral drugs required in PHA/IL-2-activated CD4<sup>+</sup> T cells, as compared with MO-DCs.

It seems logical that NNRTIs, which do not depend on kinase-mediated activation, have a similar activity in T cells and MO-DCs. Previous studies, comparing the antiviral activity of RTIs in primary monocyte-macrophages (related to DCs) and primary T cell blasts, also demonstrated that the nucleoside RTIs tended to display higher potency in the former cells, whereas NNRTIs exhibited similar activity in both cell types.<sup>42-44</sup>

Besides antiviral activity per se, timing of RTI administration is important in PEP. In our model, immediate or 24-hr-delayed treatment had a similar effect. Conversely, if RTI treatment was further postponed, viral suppression waned. The maintained drug effect during the first 24 hr probably indicated that the virus did not grow to a greater multiplicity of infection. After this 24-hr incubation period, viral replication increases, with peak infection after approximately 1 week of primary culture. Even a PEP treatment initiated as soon as 48 or 72 hr after infection was shown to be ineffective in aborting infection.

In view of the low risk of infection per sexual contact,<sup>1,2</sup> interference with induction of potentially protective immune responses must be avoided. Using MLC as a test system, we ob-

served low immune suppression with single drugs, as well as with drug combinations. These results should be confirmed in HIV antigen-specific T cell activation.

To uncover virus below the detection limit of the ELISA, PHA/IL-2-activated PBMCs were added after stopping the PEP treatment (drugs washed away).<sup>23</sup> This set-up also mimics what happens during *in vivo* immune stimulation (e.g., intercurrent infection). Although in a first experiment abortion of infection was obtained with high doses of the potent NNRTI efavirenz and/or the acyclic nucleotide phosphonate PMEA, HIV was rescued in approximately half the microcultures of second and third experiments under the same conditions. The source of the rescued HIV could be latent provirus inside the DCs or infectious virus, sticking to the surface of either DCs or autologous CD4<sup>+</sup> T cells.<sup>32,35,45</sup> In any case, the incomplete PEP effect reflects the clinical experience of protection in a proportion of exposed individuals, even if combination therapy is administered for several weeks after exposure.<sup>2,7,8</sup>

The fact that AZT was not successful in aborting infection in cultures of MO-DCs, whereas PMEA was, could be partially explained on a metabolic basis. We investigated the metabolic pathway of AZT and PMPA (an acyclic nucleoside phosphonate RTI like PMEA). Whereas AZT needs to be phosphorylated to its triphosphate form by the cell cycle-dependent thymidine kinase, PMPA needs only a dual phosphorylation by the cell cycle-independent AMP kinase. The amounts of active PMPA (PMPApp) were 30- to 45-fold higher in MO-DCs and MO-LCs as compared with the amounts of AZT-TP.<sup>46</sup> This can partially explain why compounds such as PMPA and PMEA are more efficient in aborting infection in cultures of MO-DCs than AZT. On the other hand, these metabolic studies fail to explain why PMPA and PMEA have a higher EC<sub>50</sub> than AZT.

In view of the incomplete HIV-abortive effect of present RTIs, antiretroviral drugs acting on other targets need to be tested. Protease inhibitors have the disadvantage of acting at a postintegration step.<sup>47</sup> In combination with RTIs, they can, however, be useful in preventing amplification of the infection. Integrase and fusion inhibitors are also promising candidates to act in concert with RTIs.<sup>48-50</sup> It would be useful to evaluate these compounds for their intrinsic PEP potential and for their possible additive or synergistic effect with RTIs.

In a preliminary experiment, efavirenz seemed to be more potent against two different primary NSI/R5 isolates as compared with Ba-L, used as free infecting virus. All strains were used at a multiplicity of infection of 10<sup>-3</sup>, based on TCID<sub>50</sub> values determined after 1 week of culture. However, the HIV antigen concentration was higher for Ba-L after 2 weeks of culture than for both primary isolates, pointing to a higher replicative capacity of Ba-L. This can be an alternative explanation for the differences in drug sensitivity found between primary isolates and the reference strain Ba-L. For this reason, we can only conclude that primary isolates seem at least as sensitive to inhibition by the NN-RTI efavirenz as Ba-L.

In summary, we feel that our relatively simple system can provide a useful model for exploring PEP after sexual HIV transmission and performing a first screening of various compounds and their combinations. We showed preliminary evidence that administration of potent RTIs 24 hr after exposure strongly suppresses HIV infection, without interference with normal immune function. Obviously, *in vitro*-differentiated

MO-DCs are not identical to their *in vivo* counterparts. Moreover, it is possible that other target cells, including tissue macrophages or locally activated T cells, are important in the early phase of infection. Therefore, these results need to be confirmed in other *in vitro* and *in vivo* models before extrapolations to humans can be made.

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