Functional Gene Transfer of HIV DNA by an HIV Receptor-Independent Mechanism

Anna-Lena Spetz, Bruce K. Patterson, Karin Lore, Jan Andersson and Lars Holmgren

*J Immunol* 1999; 163:736-742;

http://www.jimmunol.org/content/163/2/736

References  This article cites 50 articles, 21 of which you can access for free at:

http://www.jimmunol.org/content/163/2/736.full#ref-list-1

Why The JI? Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription  Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

Permissions  Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts  Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
HIV-1 enters target cells mainly via binding to CD4 and its coreceptors. The presence of HIV-1 in CD4+ cells suggests, however, that there exist other mechanisms for viral entry. Here it is reported that HIV-1 DNA may be transferred from one cell to another by uptake of apoptotic bodies in a CD4-independent way. This was investigated by coculturing CD4+ cells with fibroblasts harvested from HIV-1-infected patients. After 2 wk of coculture, fibroblasts contained HIV-1 DNA and expressed HIV-1 proteins p24 and gp120. Transfer of HIV-1 DNA was verified by coculturing fibroblasts with apoptotic bodies derived from cells infected with a defective HIV-1 virus. These cells contain one integrated copy of a reverse transcriptase (RT)-negative HIV-1 strain (8E5/LAV RT−) and consequently cannot produce free virus. Intracellular HIV-1 gag DNA was detected in both fibroblasts and dendritic cells after coculture with apoptotic 8E5/LAV RT− cells. Transfer of viral DNA after uptake of apoptotic bodies may explain HIV-1 infection of CD4− cells in vivo and furthermore may be relevant for Ag presentation.


Materials and Methods

Cell cultures and HIV-1 strains

Human fetal lung fibroblasts and a human endothelial cell line (EaHy 926) (33) were cultured in DMEM (HyClone Europe, Perstorp, Sweden) supplemented with 2 mM l-glutamine (Life Technologies, Taby, Sweden), penicillin and streptomycin (Life Technologies), HEPES (Life Technologies), and 10% FCS (HyClone). These cells were treated with trypsin-EDTA (Life Technologies), washed twice in PBS supplemented with 10% FCS, and transferred to Lab-Tek chamber slides (Nune, Naperville, IL; 5 × 103 cells/well) 1 day before addition of 1 × 105 apoptotic cells or cell-free primary T cell tropic virus isolates (100 tissue culture 50% infectious dose). Dendritic cells were generated from PBMC by culture in human rIL-4 (450 U/ml; Genzyme, Cambridge, MA) and GM-CSF (250 ng/ml; Leucotax, Shering-Plough, Brinny, Ireland) as previously described (34–36). Generated immature dendritic cells were used for coculture experiments on day 6 or 7. Apoptotic cells (5 × 105 cells/ml) were added to 3 × 106 dendritic cells/ml in a 24-well plate. The HIV-1 Ba-L isolate (500 tissue culture 50% infectious dose) (37), HuT78 gp2 (38), and 8E5/LAV RT− cells (39) were obtained through the AIDS Research and Reference

Copyright © 1999 by The American Association of Immunologists

0022-1767/99/$02.00

---

Anna-Lena Spetz, Bruce K. Patterson, Karin Lore, Jan Andersson, and Lars Holmgren

1 Department of Immunology, Microbiology, Pathology and Infectious Diseases, Divisions of Infectious Diseases and Virology, and Center for Genomics Research, The Karolinska Institute, Sweden; and Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, IL 60611

Received for publication March 19, 1999. Accepted for publication April 26, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 This work was supported by the Swedish Physicians Against AIDS Research Foundation, the Swedish Medical Council (Grant 10850), the National Cancer Institute (Grant 2766), the National Institutes of Health (Grant U01 AI41536-02), and the Swedish Cancer Society.

3 Address correspondence and reprint requests to Dr. Anna-Lena Spetz, Division of Infectious Diseases, The Karolinska Institute, Huddinge University Hospital, 852, S-141 86 Huddinge, Sweden. E-mail address: anna-lena.spetz@impi.ki.se

4 Abbreviations used in this paper: CCR, chemokine receptor; FISNA, fluorescent in situ 5'-nucleic acid assay; PI, propidium iodide.

---

Copyright © 1999 by The American Association of Immunologists

0022-1767/99/$02.00

---

Anna-Lena Spetz, Bruce K. Patterson, Karin Lore, Jan Andersson, and Lars Holmgren

1 Department of Immunology, Microbiology, Pathology and Infectious Diseases, Divisions of Infectious Diseases and Virology, and Center for Genomics Research, The Karolinska Institute, Sweden; and Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, IL 60611

Received for publication March 19, 1999. Accepted for publication April 26, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 This work was supported by the Swedish Physicians Against AIDS Research Foundation, the Swedish Medical Council (Grant 10850), the National Cancer Institute (Grant 2766), the National Institutes of Health (Grant U01 AI41536-02), and the Swedish Cancer Society.

3 Address correspondence and reprint requests to Dr. Anna-Lena Spetz, Division of Infectious Diseases, The Karolinska Institute, Huddinge University Hospital, 852, S-141 86 Huddinge, Sweden. E-mail address: anna-lena.spetz@impi.ki.se

4 Abbreviations used in this paper: CCR, chemokine receptor; FISNA, fluorescent in situ 5'-nucleic acid assay; PI, propidium iodide.

---

Copyright © 1999 by The American Association of Immunologists

0022-1767/99/$02.00

---

Anna-Lena Spetz, Bruce K. Patterson, Karin Lore, Jan Andersson, and Lars Holmgren

1 Department of Immunology, Microbiology, Pathology and Infectious Diseases, Divisions of Infectious Diseases and Virology, and Center for Genomics Research, The Karolinska Institute, Sweden; and Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, IL 60611

Received for publication March 19, 1999. Accepted for publication April 26, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 This work was supported by the Swedish Physicians Against AIDS Research Foundation, the Swedish Medical Council (Grant 10850), the National Cancer Institute (Grant 2766), the National Institutes of Health (Grant U01 AI41536-02), and the Swedish Cancer Society.

3 Address correspondence and reprint requests to Dr. Anna-Lena Spetz, Division of Infectious Diseases, The Karolinska Institute, Huddinge University Hospital, 852, S-141 86 Huddinge, Sweden. E-mail address: anna-lena.spetz@impi.ki.se

4 Abbreviations used in this paper: CCR, chemokine receptor; FISNA, fluorescent in situ 5'-nucleic acid assay; PI, propidium iodide.

---

Copyright © 1999 by The American Association of Immunologists

0022-1767/99/$02.00

---

Anna-Lena Spetz, Bruce K. Patterson, Karin Lore, Jan Andersson, and Lars Holmgren

1 Department of Immunology, Microbiology, Pathology and Infectious Diseases, Divisions of Infectious Diseases and Virology, and Center for Genomics Research, The Karolinska Institute, Sweden; and Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, IL 60611

Received for publication March 19, 1999. Accepted for publication April 26, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 This work was supported by the Swedish Physicians Against AIDS Research Foundation, the Swedish Medical Council (Grant 10850), the National Cancer Institute (Grant 2766), the National Institutes of Health (Grant U01 AI41536-02), and the Swedish Cancer Society.

3 Address correspondence and reprint requests to Dr. Anna-Lena Spetz, Division of Infectious Diseases, The Karolinska Institute, Huddinge University Hospital, 852, S-141 86 Huddinge, Sweden. E-mail address: anna-lena.spetz@impi.ki.se

4 Abbreviations used in this paper: CCR, chemokine receptor; FISNA, fluorescent in situ 5'-nucleic acid assay; PI, propidium iodide.

---

Copyright © 1999 by The American Association of Immunologists

0022-1767/99/$02.00

---

Anna-Lena Spetz, Bruce K. Patterson, Karin Lore, Jan Andersson, and Lars Holmgren

1 Department of Immunology, Microbiology, Pathology and Infectious Diseases, Divisions of Infectious Diseases and Virology, and Center for Genomics Research, The Karolinska Institute, Sweden; and Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, IL 60611

Received for publication March 19, 1999. Accepted for publication April 26, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 This work was supported by the Swedish Physicians Against AIDS Research Foundation, the Swedish Medical Council (Grant 10850), the National Cancer Institute (Grant 2766), the National Institutes of Health (Grant U01 AI41536-02), and the Swedish Cancer Society.

3 Address correspondence and reprint requests to Dr. Anna-Lena Spetz, Division of Infectious Diseases, The Karolinska Institute, Huddinge University Hospital, 852, S-141 86 Huddinge, Sweden. E-mail address: anna-lena.spetz@impi.ki.se

4 Abbreviations used in this paper: CCR, chemokine receptor; FISNA, fluorescent in situ 5'-nucleic acid assay; PI, propidium iodide.
Reagent Program, National Institutes of Health (McKesson BioServices, Rockville, MD), and HuT78 cells were obtained from American Type Culture Collection (Manassas, VA).

Viral RNA was extracted from PE-infected patients or HIV-1-seronegative blood donors were isolated from EDTA-blood by density centrifugation on Ficoll-Hypaque gradients (Pharmacia, Upssala, Sweden). Plasma HIV-1 viremia was measured by a branch DNA assay (Chiron, Emeryville, CA). CD4 T cell counts were performed by routine clinical laboratory testing. Apoptosis was induced either by gamma irradiation (150 Gy) 1–3 h before addition to the cultures or by treatment with etoposide 16 μg/ml for 48 h.

**Immunofluorescence**

Stainings were performed as previously described (40). In brief, cells were washed with PBS before fixation in 3.7% paraformaldehyde in PBS for 10 min. To reduce nonspecific Ab binding, cells were first incubated with 2% FCS in Earle’s balanced salt solution (Life Technologies) supplemented with 0.01 M HEPES buffer (Life Technologies). Cells were then permeabilized with 0.1% saponin dissolved in balanced salt solution to allow intracellular entrance of HIV-1-specific Abs. To prevent unspecific binding of secondary Abs, 1% goat serum (Dako, Glostrup, Denmark) was added during incubation with primary Abs. Primary Abs diluted in balanced salt solution-saponin were added and left to incubate for 45 min at 37°C. After several washes with balanced salt-saponin the secondary Ab and Hoechst 33258 (Sigma, Stockholm, Sweden) were added and left to incubate at room temperature. Cells were examined in a Leica RXM microscope (Leica, Wetzlar, Germany). The following mouse mAbs were used: anti-p24 (KAL-1, IgG1, Dako), anti-gp120 (8835, IgG1, Chemicon, Temecula, CA), anti-CXCR4 (12G5, IgG2a, PharMingen, San Diego, CA), anti-CCR5 (2D7, IgG2a, PharMingen), anti-CD4 (IgG1, Becton Dickinson, San Diego, CA), and anti-vimentin (Dako). Secondary goat anti-mouse Abs were Oregon Green-conjugated anti-Ig (Molecular Probes, Eugene, OR).

**Quantification of cell HIV-1 DNA content**

Adherent cells (5 x 10⁴ cells/sample) were trypsinized and washed in PBS twice before fixation in Permeafix (Ortho Diagnostics, Raritan, NJ). Nonadherent cells (1 x 10⁴ cells/sample) were washed in PBS and thereafter stored in Permeafix. HIV-1 gag DNA was detected by a fluorescent in situ hybridization assay (FISNA) (41, 42). The PCR was performed in cell suspension (1 x PCR buffer II; 0.35 mM MgCl₂; 200 μM each of dATP, dGTP, dCTP, dTTP; 200 μM each of gag primers SK38/SK39, sequence 5’-ATAATCCACCTACCTGAGGAGAAAT-3’ and 5’-TTTGTGCTCTTGTCTTATGTCCAGAATGC-3’; 0.1 μM of FAM-labeled quenching dye), sequence 5’-FTSK19 (FAM served as the reporter dye and TAMRA served as the quencher dye), sequence 5’-ATAATCCACCTACCTGAGGAGAAAT-3’ and 100 nM of gag probe sequences 5’-ATAATCCACCTACCTGAGGAGAAAT-3’, 20°C. Quantitative kinetic RT-PCR (43) was performed by using the Gene Amp PCR System 2400 (PE Applied Biosystems, Foster City, CA). Reaction tubes were heated to 95°C for 5 min followed by 30 cycles of 94°C for 45 s and 56°C for 2 min, followed by a 15°C soak.

**Flow cytometry**

Irradiated or etoposide-treated PBMC, HuT78, or 8E5/LAV RT⁺ cells were stained with annexin V-FTTC (Boehringer Mannheim, Mannheim, Germany) and propidium iodide (PI) according to the manufacturer’s protocol. Early apoptosis was defined by annexin V⁺ PI⁻ staining as determined by FACScan or FACSCalibur (Becton Dickinson). The kinetics of cell death after irradiation (2, 4, 10, 18, 24, 48 h) or etoposide treatment (12, 24, 48 h) were studied in noninfected cells, since HIV-1-infected cells were always fixed in paraformaldehyde before analyses by flow cytometry. Fluorescence intensity was measured using a log₁₀ scale, and 10,000 events were analyzed per sample.

**Results**

**Transfer of HIV-1 DNA to fibroblasts**

Human fetal fibroblasts that lacked detectable mRNA and protein expression of CD4, CCR5, and CXCR4, as shown by immunofluorescent stainings and quantitative kinetic RT-PCR (Table I and data not shown), were used to study HIV-1 receptor-independent transfer of HIV-1. Freshly isolated PBMC or HuT78 cells were used as positive controls for immunofluorescent stainings of CD4, CCR5, and CXCR4, while macrophages (adherent PBMC cultured for 1 wk) were used as a positive control for the expression of CCR5 and CXCR4 mRNA (Table I). To investigate whether HIV-1 DNA could be transferred by the uptake of apoptotic bodies in coculture experiments, HIV-1-infected and noninfected T cell lymphomas as well as PBMC were induced to undergo apoptosis before addition to fibroblast cultures. Apoptosis, as detected by annexin V binding was induced by either gamma irradiation (150 Gy) or treatment with etoposide (Fig. 1). Freshly isolated PBMC contained some debris and dead cells that were annexin V⁺ PI⁻ and a few cells bound annexin V but did not take up PI (Fig. 1A). HIV-1-infected T cell lymphomas contained about 10–20% annexin-V⁺ debris and cells before induction of apoptosis (Fig. 1B). Almost all HIV-1-infected T lymphomas (Fig. 1, D and F) and around 50% of PBMC (Fig. 1, C and E) bound annexin V after 48 h of etoposide treatment or 18–24 h after irradiation. Approximately 15–20% of PBMC also took up PI, a sign of secondary necrosis. Fibroblasts were cocultured with apoptotic HuT78SF2⁺ or noninfected HuT78 cells for 1 or 2 wk as well as adherent PBMC (macrophages) cultured in RPMI-10% FCS for 1 wk, by Trizol reagent (Life Technologies) according to the manufacturer’s protocol. The cells were trypsinized in TrypLE EZ buffer (PE Applied Biosystems, Foster City, CA), 4.0 mmol/L Mn(O)Ac₂, 300 μmol/L dCTP, dGTP, and dTTP; 200 μmol/L upstream primer, 200 nmol/L downstream primer, 200 nmol/L internally conserved fluorogenic probes, and 10 U of TTH polymerase) directly to 100 ng of total RNA in 5 μl of RNase- and DNasefree water (Ambion, Austin, TX). RNA was normalized using glyceraldehyde-3-phospho-
released from apoptotic HuT78 SF2 cells, cocultures with apoptotic bodies derived from a T lymphoma infected with a defective virus were analyzed. 8E5/LAV RT cells contain one integrated copy of an HIV-1 strain that lacks RT and consequently cannot produce complete viral particles, but can produce some HIV-1-encoded proteins (39). Culture supernatants from 8E5/LAV RT cells were analyzed by a sensitive RT assay at several time points to assure that the cells lacked RT (data not shown). Fibroblasts cocultured up to 8 wk with apoptotic bodies from 8E5/LAV RT cells contained gag DNA, showing that the intracellular presence of HIV-1 DNA was not due to infection with viral particles derived from the apoptotic T lymphomas (Fig. 2a).

To exclude that HIV-1 detected in fibroblasts were due to remaining apoptotic bodies, the expressions of CXCR4 and CCR5 mRNA, originating from HuT78 cells, were followed by quantitative kinetic RT-PCR. Fibroblasts cocultured up to 8 wk with apoptotic bodies from 8E5/LAV RT cells contained gag DNA, showing that the intracellular presence of HIV-1 DNA was not due to infection with viral particles derived from the apoptotic T lymphomas (Fig. 2a).

To exclude that HIV-1 detected in fibroblasts were due to remaining apoptotic bodies, the expressions of CXCR4 and CCR5 mRNA, originating from HuT78 cells, were followed by quantitative kinetic RT-PCR. Fibroblasts cocultured with apoptotic bodies from 8E5/LAV RT cells contained gag DNA, showing that the intracellular presence of HIV-1 DNA was not due to infection with viral particles derived from the apoptotic T lymphomas (Fig. 2a).

Table I. Human fetal lung fibroblasts lack expression of CCR5 and CXCR4 mRNA

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR5 mRNA(a)</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Fibroblast + apoptotic 8E5/LAV RT()</td>
<td>0.1</td>
<td>0.2</td>
<td>71</td>
<td>24</td>
</tr>
<tr>
<td>Fibroblast + apoptotic HuT78sf2</td>
<td>0.2</td>
<td>0.2</td>
<td>247</td>
<td>51</td>
</tr>
<tr>
<td>Fibroblast + apoptotic noninfected HuT78</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fibroblast + apoptotic 8E5/LAV RT()</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fibroblast + apoptotic HuT78sf2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fibroblast + apoptotic noninfected HuT78</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Macrophages(b)</td>
<td>66</td>
<td>13</td>
<td>1966</td>
<td>537</td>
</tr>
</tbody>
</table>

\(a\) Human fetal lung fibroblasts were transferred to Lab-Tek chamber slides (5 × 10\(^5\) cells/well) before addition of apoptotic (1 × 10\(^5\) cells/well) 8E5/LAV RT\(\) T cells infected with a defective RT\(\) HIV-1 isolate, or apoptotic HIV-1-infected HuT78sf2 T cells or apoptotic noninfected HuT78 T cells. After 1 or 2 wk of coculture, chemokine receptor mRNA was quantified by RT-PCR. Values (mean and SD from duplicates) equal number of chemokine receptor mRNA copies per 10,000 G3PDH mRNA copies.

\(b\) PBMC were cultured for 1 wk. The recovered adherent cells were analyzed for CCR5 and CXCR4 expression and used as positive control.

HIV-1 p24 and gp120 detected in fibroblasts after uptake of apoptotic bodies

To investigate whether the transferred HIV-1 DNA was transcribed, fibroblasts were analyzed for protein expression of HIV-1 p24 and gp120 Ags. Immunofluorescent labelings showed expression of the HIV-1-encoded gene products p24 and gp120 in fibroblasts after 2 wk of coculture with apoptotic 8E5/LAV RT\(\) and apoptotic HuT78sf2 cells (Fig. 2b). The staining pattern was characterized by the accumulation of protein in the cytosol. Fibroblasts cocultured with noninfected HuT78 cells did not express p24 or gp120 as expected (Fig. 2b). The frequency of p24 Ag-positive fibroblasts after coculture with apoptotic 8E5/LAV RT\(\) and apoptotic HuT78sf2 cells ranged between 0.3–1.7% in five independent experiments.
Dendritic cells can present Ag derived from apoptotic cells, stimulating MHC class I-restricted Ag-specific CD8⁻ cytotoxic T cells (44). We therefore investigated whether HIV-1 DNA could be transferred to dendritic cells by uptake of apoptotic bodies. Dendritic cells express HIV-1 receptors (45), an expression pattern that seems to be tightly regulated during dendritic cell maturation (46). Apoptotic 8E5/LAV RT⁻ cells infected with the defective, RT⁻ virus were therefore used in cocultures with dendritic cells. Dendritic cells were prepared from peripheral blood precursors of healthy donors by in vitro culture in the presence of rIL-4 and GM-CSF (34–36). Apoptotic 8E5/LAV RT⁻ cells were added to the in vitro differentiated dendritic cells after 6–7 days of culture. At this time dendritic cells were CD14⁻, HLA-DR⁻, CD83⁻, and CD86⁻ (36), characteristic of an immature phenotype with phagocytosing capacity (47). HIV-1 gag DNA could be detected by FISNA in approximately 18% of dendritic cells after 2 wk of coculture with apoptotic 8E5/LAV RT⁻ cells and in 9% after infection with a cell-free macrophage-tropic Ba-L isolate.
and Table II). Dendritic cells cocultured with noninfected cells or without any virus isolates did not emit positive signals for gag DNA. These results show that HIV-1 DNA can be transferred to dendritic cells by uptake of apoptotic bodies.

**Transfer of HIV DNA by apoptotic HIV-1-infected PBMC**

PBMC from HIV-1-infected patients contain cells that are latently infected and in which the viral cDNA is integrated within host cell DNA (3). To assess whether PBMC isolated from HIV-1-infected patients could transfer HIV-1 by uptake of apoptotic bodies, fibroblasts were cocultured with apoptotic PBMC isolated from HIV-1-infected patients. PBMC were isolated from five patients with HIV RNA levels of <2.7–6.5 log_{10} copies/ml of plasma and CD4 cell counts between 25–220/mm³. Fibroblasts cocultured with apoptotic bodies derived from PBMC isolated from HIV-1-infected patients contained gag DNA after 2–8 wk of culture at a frequency of 6–51% (Fig. 3c and Table II). Freshly isolated PBMC (from the same HIV-1-infected patients) that had not been induced to undergo apoptosis by irradiation as well as apoptotic PBMC from HIV-1-seronegative donors did not transfer HIV-1 DNA to cocultured fibroblasts (Fig. 3c and Table II).

Fibroblasts cocultured with apoptotic PBMC isolated from HIV-1-infected patients also expressed the HIV-1 Ag p24 (Fig. 3b). The frequency of intracellular p24 Ag-positive fibroblasts detected by immunofluorescence after 2 wk of coculture with apoptotic PBMC isolated from HIV-1-infected patients ranged between 0.6–2.7%, thus similar to the frequency detected in cocultures with apoptotic 8E5/LAV RT⁻ and apoptotic HuT78SF2 cells (0.3–1.7%).

**Discussion**

In the present study it was shown that fibroblasts and dendritic cells cocultured with apoptotic bodies derived from HIV-1-infected cells, resulted in uptake of HIV-1 DNA and expression of HIV-1 proteins p24 and gp120. Two lines of evidence indicated that the transfer of HIV-1 was CD4-, CCR5-, and CXCR4-independent. First, the fibroblasts used in these experiments did not express detectable mRNA or protein levels of CD4, CCR5, or CXCR4. Second, transfer of HIV-1 DNA was possible using apoptotic bodies from HIV-1-infected T lymphoma with one integrated copy of a defective RT⁻ virus. This defective RT⁻ virus cannot produce HIV-1 viral particles (39). The results presented here, hence, are consistent with transfer of HIV-1 DNA to the fibroblasts and dendritic cells. The transferred HIV-1 DNA was, furthermore, transcriptional and resulted in expression of HIV-1-encoded proteins p24 and gp120.

The in vitro infectibility of peripheral CD8⁻ T cells from adults was shown to be dependent on the presence of CD4⁺ T cells in the initial culture exposed to HIV-1 (12). The CD8⁻ T cells were also infected with HIV-1 after coculture with autologous HIV-1-infected CD4⁺ T cells (12). CD8⁻ cytotoxic T cells were, moreover, shown to become infected in vitro in the process of killing HIV-1-infected target cells, a process involving apoptosis of the infected target cell (48). It remains to be elucidated whether the infection of CD8⁻ T cells was caused by transfer of HIV DNA after uptake of apoptotic bodies derived from either CD4⁺ T cells or HIV-1-infected target cells.

CD4⁻ cells infected with HIV-1 have been observed at several anatomic locations in HIV-1-infected individuals (6–8, 17–19). Findings presented here raise the question of whether apoptotic bodies derived from HIV-1-infected cells can transfer viral DNA to HIV-1 receptor-negative, phagocytes in cells in vivo. Such a mechanism could play a role in virus persistence in the infected individual and may explain the spread of HIV-1 to, for example, endothelial cells in the brain as well as epithelial cells. The pathogenesis of HIV-1 infection is characterized by increased frequency of apoptosis (49). The finding that PBMC isolated from HIV-1-infected patients could transfer HIV-1 DNA supports the hypothesis that viral transfer by apoptotic bodies could also play a role in vivo. It was, however, necessary to induce apoptosis in the infected PBMC, since freshly isolated PBMC could not transfer HIV-1 to

**Table II. Detection of HIV-1 DNA in cocultures by FISNA**

<table>
<thead>
<tr>
<th>Cocultures</th>
<th>FISNA Detection 2-wk coculture</th>
<th>FISNA Detection 8-wk coculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast + apoptotic 8E5/LAV RT⁻</td>
<td>19</td>
<td>5.2</td>
</tr>
<tr>
<td>Fibroblast + apoptotic HuT78SF2</td>
<td>46</td>
<td>68</td>
</tr>
<tr>
<td>Fibroblast + apoptotic noninfected HuT78</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Fibroblast + apoptotic PBMC HIV-1-infected donor</td>
<td>51</td>
<td>7.1</td>
</tr>
<tr>
<td>Fibroblast + apoptotic PBMC seronegative donor</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Fibroblast + T cell tropic primary virus isolate</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Dendritic cells + apoptotic 8E5/LAV RT⁻</td>
<td>18</td>
<td>ND</td>
</tr>
<tr>
<td>Dendritic cells + apoptotic noninfected HuT78</td>
<td>0.1</td>
<td>ND</td>
</tr>
<tr>
<td>Dendritic cells + Ba-L isolate</td>
<td>8.9</td>
<td>ND</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>0.1</td>
<td>ND</td>
</tr>
<tr>
<td>Endothelial + apoptotic 8E5/LAV RT⁻</td>
<td>1.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Endothelial + apoptotic noninfected HuT78</td>
<td>0.1</td>
<td>ND</td>
</tr>
<tr>
<td>Endothelial + apoptotic PBMC HIV-1-infected donor</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Endothelial + apoptotic PBMC seronegative donor</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Endothelial + T cell tropic primary virus isolate</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Notes:

- HIV-1 DNA was detected by FISNA (41). The cells were evaluated for presence of HIV-1 DNA at the single cell level by an ACAS 570 laser confocal microscope. Values above 1.5% are considered positive. One representative experiment of at least two is shown.
- Human fetal lung fibroblasts and human endothelial cells were transferred to Lab-Tek chamber slides (5 × 10⁴ cells/well) 1 day before addition of indicated apoptotic cells (1 × 10⁴ cells/well) or cell-free virus isolates (10 TCID₅₀). Dendritic cells were generated from PBMC from blood donors by culture in medium containing human rIL-4 and GM-CSF. Generated immature dendritic cells were used for coculture experiments at day 6 or 7. Apoptotic cells (5 × 10⁴ cells/ml) were added to 3 × 10⁴ dendritic cells/ml. HIV-infected HuT78SF2 (38), noninfected HuT78, 8E5/LAV RT⁻ cells (39), and PBMC isolated from HIV-1-infected patients or healthy blood donors, were irradiated 150 Gy, before addition to the cultures.
HIV-1 receptor-negative cells. This suggests that apoptotic PBMC may transfer HIV-1 DNA to CD4+ cells in vivo.

Dendritic cells have been shown to acquire Ag from apoptotic cells and induce MHC class I-restricted CTL (44) as well as present phagocytosed cellular fragments on MHC class II molecules (50). The source(s) of the peptides presented by MHC molecules remains unresolved. Are they derived from processed phagocytosed proteins that have been stored in dendritic cells after internalization of apoptotic bodies (50), and/or do they derive from endogenously produced peptides after transfer of viral DNA? Follicular dendritic cells have been shown to be able to present Ag for long periods of time after Ag exposure (51, 52). In the current study transferred HIV DNA could be detected in high frequencies for up to 8 wk after initiation of cocultures. We speculate that transfer of DNA, leading to expression of proteins and processing of peptides in the APC, could account for prolonged capacity of Ag presentation.

Acknowledgments

We thank Lena Radler for expert technical assistance, Margit Halvarsson and Ann-Sofie Löfstrand for blood samples, and Dr. Anders Sönnerborg (Department of Clinical Virology, Huddinge Hospital) for help and advice.

References
