View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Kidney International, Vol. 53 (1998), pp. 1217–1229

Infection of human primary renal epithelial cells with HIV-1 from children with HIV-associated nephropathy

Patricio E. Ray, Xue-Hui Liu, Dot Henry, Louis Dye III, Lian Xu, Jan M. Orenstein, and Ted E. Schuztbank

Children's Research Institute, Research Center IV and Department of Laboratory Medicine, Children's National Medical Center, and Department of Pathology, The George Washington University, Washington, D.C., USA

Infection of human primary renal epithelial cells with HIV-1 from children with HIV-associated nephropathy. Children affected with human immunodefficiency virus (HIV)-associated nephropathy (HIVAN) usually develop significant renal glomerular and tubular epithelial cell injury. The pathogenesis of these changes is not clearly understood. Human renal tubular epithelial cells (RTEc) do not express CD4 surface receptors, and it is not clear whether these cells can be infected by HIV-1. Certain strains of HIV-1, however, have been shown capable of infecting CD4-negative epithelial cell lines. We hypothesized that the inability of laboratory strains of HIV-1 to infect renal epithelial cells may be due to a limited tropism, as opposed to wild-type viruses derived from children with HIVAN, and that viruses derived from these children are capable of infecting RTEc from the same patient. Here, we have demonstrated that HIV-1 isolates from children with HIVAN can productively infect RTEc through a CD4 independent pathway, and that infected mononuclear cells can transfer the virus to human RTEc. Human RTEc sustained low levels of viral replication and HIV-1 inhibited the growth and survival of cultured human RTEc. Thus, HIV-1 may directly induce degenerative changes in RTEc of children with HIVAN. Infected macrophages may play a relevant role in this process by transferring viruses to RTEc.

Human immunodeficiency virus (HIV)-associated nephropathy (HIVAN) is a clinicopathologic entity that includes proteinuria, focal segmental or global glomerulosclerosis and tubulointerstitial disease [1–5]. The prevalence of HIVAN is highest among African-Americans [6, 7]. While no single morphological feature is specific of HIVAN, the swelling of glomerular visceral epithelial cells and severe tubular microcystic dilation leading to kidney enlargement are prominent findings of HIVAN [1–5, 8]. Although the pathogenesis of these changes is unknown, HIV-1 may have direct cytotoxicity by infecting renal epithelium or by inducing the release of cytokines, viral proteins, or a combination of any of the above. Studies in HIV-1 transgenic mice support the hypothesis that HIV-1 gene products can injure renal epithelium even in the absence of immunosupression or productive viral infection [9–11]. Human studies attempting to detect a productive infection of HIV-1 in human glomerular and tubular epithelium, however, have been inconclusive [12–15].

Human renal epithelium does not express CD4 receptors, and *in vitro* attempts to infect glomerular epithelial cells using laboratory strains of HIV-1 have proven fruitless [16]. Nevertheless, certain strains of HIV-1 have been shown capable of infecting CD4-negative human cells including fibroblasts [17, 18], neural cells [19, 20], transformed colonic epithelial cells [21, 22], trophoblast [23] and cervical epithelial cells (ME180) [24–26]. These studies indicate that other molecules can facilitate HIV-1 infection in CD4-negative cells [27–29]. A G protein-coupled receptor named CXCR4 [30] and the chemokine receptors CCR5, CCR2b and CCR3 [31–33] are recently described co-factors necessary for viral entry even in the presence of the CD4 receptor and may play a role in HIV-1 infection of CD4-negative cells. Thus, it is possible that HIV-1 may adapt to host tissues by using cell surface receptors or by acquiring adhesion molecules from host cells [29] and gaining a selective tropism to infect these cells.

We hypothesized that the inability of HIV-1 to infect renal epithelial cells may be due to a limited tropism of cloned laboratory strains, as opposed to wild-type virus, and that HIV-1 isolates derived from children with HIVAN are capable of infecting renal tubular epithelial cells derived from the same patient. This study demonstrates that HIV-1 can infect renal epithelial cells and supports the hypothesis that HIV-1 may cause direct cytotoxicity of renal epithelial cells from children with HIVAN.

METHODS

Isolation of primary HIV-1 viruses

Primary HIV-1 isolates were initially derived from peripheral blood mononuclear cells (PBMCs), from three patients and from the peritoneal fluid of one additional patient after informed consent was obtained (Table 1). HIV-1 (SP-PF1) and (BS-3) were isolated from two children with HIVAN also affected with hemolytic uremic syndrome (HIVAN-HUS). HIV-1 (RW-2) was isolated from a child with HIV-HUS, and DC-4 from a child with HIVAN. All children with HUS were in renal failure. This study was approved by the Children's Hospital Institutional Review Board. PBMCs were separated on Ficoll-Hypaque gradients and propagated in 10 ml RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum, 5% interleukin-2 and 5 μg/ml

Key words: virus transmission, glomerular injury, cytotoxicity, tissue mononuclear cells, epithelial cells.

Received for publication October 3, 1997
and in revised form December 10, 1997
Accepted for publication December 10, 1997

© 1998 by the International Society of Nephrology

Table 1. Primary viral isolates used in this study

HIV-1 isolate	Source	Patient age/sex/race
SP-PF1	Peritoneal fluid	6 m/F/AA
RW-2	PBMCs	12 Y/M/C
BS-3	PBMCs	6 m/F/AA
DC-4	PBMCs	8 m/M/AA

Abbreviations are: m, months; Y, years; M, male; F, female; AA, African American; C, Caucasian, PBMCs, peripheral blood mononuclear cells.

PHA from Cellular Products (Buffalo, NY, USA). PBMCs (1×10^7) and peritoneal cells (5×10^6) were co-cultured with 1×10^7 mitogen-stimulated normal human PBMCs from healthy HIV-1 negative donors. The culture media was changed every three days. All cultures were fed with fresh HIV-1 negative PBMC (5×10^6) once a week. The HIV-1 III_B isolate from Dr. Robert Gallo [37] was obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, NIH, Bethesda, MD, USA). Samples from infected PBMCs and cell-free supernatants were frozen and saved. Virus supernatants were harvested from infected PBMCs and titrated simultaneously by limiting dilution on HIV-1 negative PBMCs. The viabilities of these cultures were monitored by vital dye exclusion as described previously by Pise, Newburger and Holland [38]. Only, cultures yielding infectious titers of $> 10^6$ $TCID_{50}$/ml were used.

Renal tubular epithelial cell cultures

Primary renal tubular epithelial cells (RTEc) were isolated from renal biopsies, autopsies, and urine of five children with HIV-associated renal disease. RTEc cultures were established as described previously [34], by cloning homogeneous micro-colonies of RTEc on collagen I-coated dishes. RTEc exhibit a characteristic epithelial morphology, growing in cobblestone-like monolayers (Fig. 1). They lack Factor VIII-related antigen, characteristic of endothelial cells, and express cytokeratin, γ-GTP, or other markers of cell differentiation depending on each specific RTEc type [34]. To preferentially enhance the growth of proximal tubular cells, which are capable of endocytosis, a potential mechanism of HIV-1 viral entry into the cells, all primary colonies were plated on collagen-coated plastic dishes and grown in the following selective renal proximal tubule culture medium: Click RPMI (Quality Biological Inc. Gaithersburg, MD, USA), supplemented with 1% fetal bovine serum (Lot #A9234J; Gemini Bioproducts, Calabasas, CA, USA), 5 ml/500 ml (100 xpen/strep), 1 mM Hepes, 2 mM L-glutamine, insulin (5 μg/ml), transferrin (5 μg/ml), and dexamethasone 5×10^{-8} M, obtained from Sigma (St. Louis, MO, USA). Insulin was omitted in collecting tubule culture media. All cells were used between passages 1 and 3 while retaining epithelial characteristics. RTEc were also isolated from HIV-negative children (Table 2). The diagnosis of HIV infection was excluded based on the clinical history and HIV-1 serology. HIV-1 serology, however, was not available in two patients. All RTEc derived from children not infected with HIV-1 were screened by HIV-1 PCR and p24 antigen measurements in culture supernatants. RTEc derived from proximal, thick ascending limb of Henle, and distal convoluted tubules were generously provided by Dr. Patricia Wilson (Mount Sinai Medical Center, New York, NY, USA). Mouse primary RTEc were isolated and grown as previously described [11]. The non permissive renal canine epithelial cell line MDCK was obtained from ATCC (Rockville, MD, USA). The HeLa-CD4+ cell clone 1022 from Dr. Bruce Chesebro [35] was obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, NIH, Bethesda, MD, USA). This adherent cell line was used as a positive control since HIV-1 III_B and many primary HIV isolates derived from patients PBMC are capable of infecting these cells [36].

To determine whether RTEc derived from HIV-infected children were not previously infected at the outset of the experiments, the following studies were performed: (*1*) polymerase chain reaction (PCR) for HIV-1 proviral DNA; (*2*) p24 antigen measurements in culture supernatants (p24 antigen values $>$ 2.0 SD above the mean from all control uninfected RTEc cells were considered positive); (*3*) determined whether HIV-1 could be rescued by co-culturing a representative sample of cells from each clone with stimulated human HIV-1 negative PBMCs; (*4*) studied selected RTEc clones by electron microscopy to exclude the presence of viral particles. RTEc derived from HIV-infected children were not used in other experiments if at least one of these results was positive.

Experimental design

Each RTEc clone or cell type was divided in two groups. One group of cells was used as control and the other group was exposed to HIV-1. In cell-associated infection experiments, RTEc were exposed to HIV-1 infected PBMCs. In cell-free infection experiments, RTEc were exposed to HIV-1 infected supernatants derived from infected PBMCs. Supernatants were filtered through a 0.4 μm pore size membrane. Control cells were exposed to stimulated HIV-1 negative PBMCs or uninfected supernatants. All experiments were done in triplicate.

In typical experiments, human RTEc were plated on 24-well plates, six-well plates, or T25 flasks, at a density of approximately 2,500 cell/cm^2. RTEc were grown until they were confluent. In cell-associated infection experiments, RTEc were exposed to HIV-1 infected PBMCs (approximately 2.4×10^3 cells/cm^2), while in cell-free infection experiments, RTEc were exposed to HIV-1 infected supernatants [$> 1 \times 10^7$ infection units (IU)/ml]. Since we did not use dextran or polybrene to facilitate the infection process, high viral titers were used, ranging from 1 to 10 MOIs, in all experiments unless indicated otherwise. RTEc were exposed to HIV-1 for 24 to 48 hours. Cells were then washed three times to remove the input virus and refed with fresh culture media. Cultures were followed for a minimum period of three weeks. Two additional washes and culture media changes were done during this period. At the end of each experiment, all cells were washed and harvested for HIV-1 studies (see infection criteria below), or co-cultured with HIV-1 negative PBMCs to determine whether HIV-1 could be rescued. Supernatants from control and HIV-exposed RTEc were used to infect normal PBMCs. To eliminate the possibility that adherent viruses were being detected, non-permissive epithelial cells (MDCK and mouse renal tubular epithelial cells) were exposed to a similar number of infectious units in both cell free and cell-associated infection experiments. In addition, the HIV-1 III_B isolate, which does not infect human epithelial cells, was used as another control to investigate whether adherent viruses could be rescued from the cell surface of human RTEc by PBMCs.

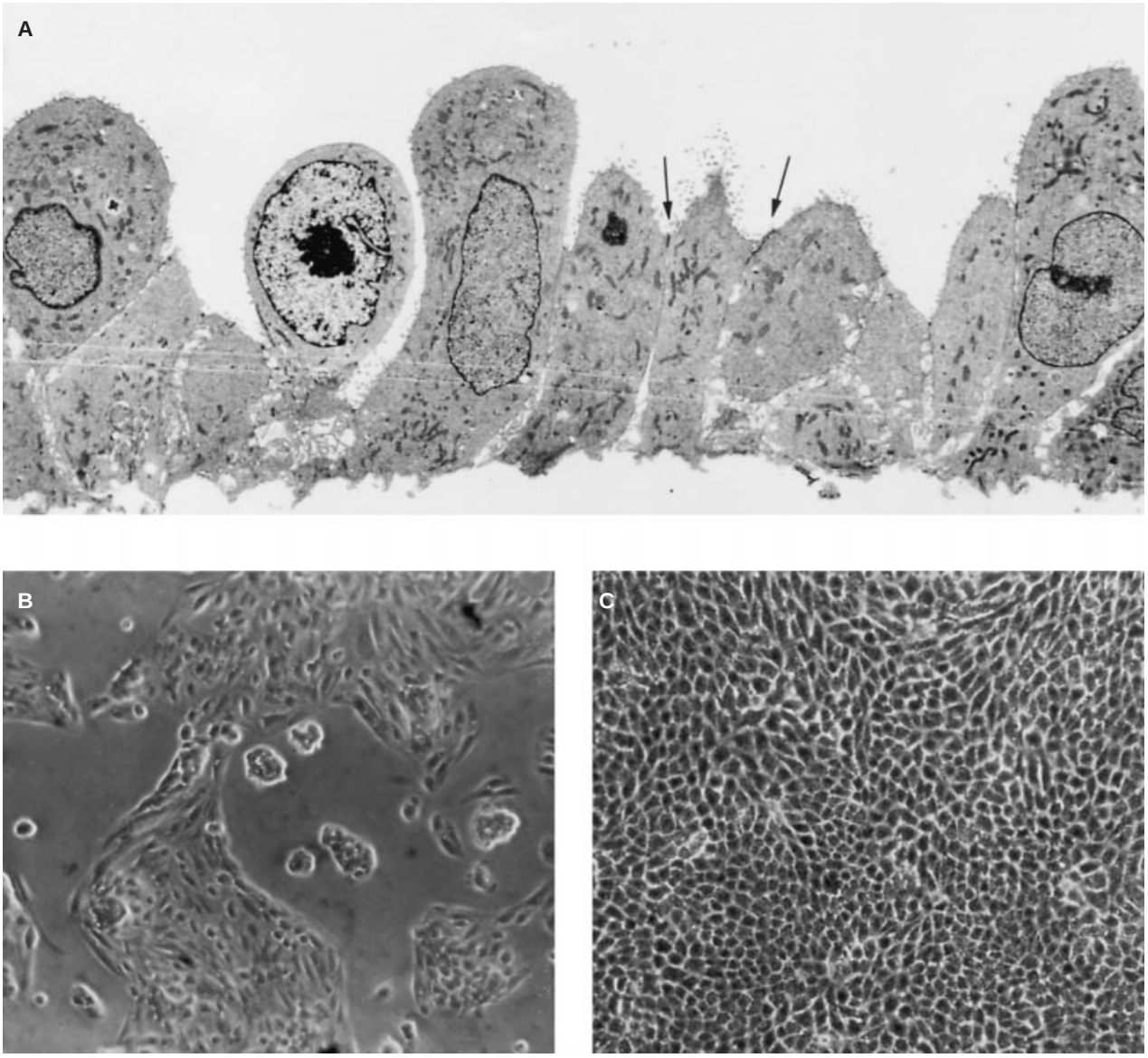


Fig. 1. A. Transmission electron microscopy showing maintenance of orientation, intercellular junctions (arrows) and microvillous surface of cultured RPTEc (×2,500). (*B and C*) Renal tubular epithelial cells (RTEc) isolated from children with HIVAN (×160).

Criteria to define HIV-1 infection

The following criteria were used to define RTEc infection: (*1*) at least two consecutive samples with absorbance values > 2.0 SD above the p24 antigen control values, and one cutoff p24 antigen value of > 12.5 pg/ml after removal of input virus; (*2*) two consecutive measurements detecting an increase in p24 antigen values in the culture media after each wash; (*3*) positive HIV proviral DNA by PCR in RTEc cultured for more than two weeks. Positive cultures were defined by a cutoff value of > 800 ECL units/1 × 10^6 cells (> 2.0 SD above control values). (*4*) Positive HIV RNA by nucleic acid sequence based amplification (NASBA) in mRNA extracted from RTEc cultured for more than two weeks. Positive cultures were defined by a cutoff value of > 700 copies/1 × 10^6 (>2.0 SD above control values). (*5*) Infection of HIV-1 negative PBMCs co-cultivated with RTEc previously exposed to HIV-1 and grown in culture for more than twenty days. All five criteria were required in both cell-free infection and cell-associated infection experiments, and in all experiments using RTEc isolated from HIV-infected or HIV negative children. To confirm the presence of positive cytoplasmic HIV-1 staining in RTEc infected in cell-associated experiments, we performed immunohistochemistry and *in situ* hybridization studies. Co-localization experiments were done using HIV-1 probes and immunohistochemical markers for mononuclear and epithelial cells by combining *in situ* hybridization and immunohistochemical techniques. Finally, we performed transmission electron microscopy (TEM) studies to confirm the presence of viral particles inside RTEc.

For blocking experiments, human renal proximal tubular epithelial cells (RPTEc) were seeded at a density of 1 × 10^6 cells per well. After 24 hours RPTEc were preincubated with anti-CD4 monoclonal antibodies, OKT4 from Ortho Diagnostics Systems

Table 2. Summary of all primary epithelial cell cultures used in this study

Cell name	Type[a]	Source	Patient[a] Age/Sex/Race	HIV-1 Status	Diagnosis[b]	Permissive for HIV-1 Infection
RW-U1	HRTEc	Urine	12y/M/C	Pos	HIV-1-HUS	Yes
RW-B1	HRPTEc	Renal biopsy	12y/M/C	Pos	HIV-1-HUS	Yes
SB-U1	HRTEc	Urine	6m/F/AA	Pos	HIVAN/HUS	Yes
SB-A1	HRTEc	Autopsy	6m/F/AA	Pos	HIVAN/HUS	Yes
DC-U1	HRTEc	Urine	8m/M/AA	Pos	HIVAN	Yes
DC-U2	HRTEc	Urine	8m/M/AA	Pos	HIVAN	Yes
RT-1	HRTEc	Renal surgery	5y/M/C	Neg	Renal malformation	Yes
RPTE-1	HRPTEc	Cadaveric kidney	19y/M/C	Neg	Unknown	Yes
RPTE-2	HRPTEc	Cadaveric kidney	19y/M/Unk	Neg	Unknown	Yes
TAL-1	HTAL	Cadaveric kidney	Unknown	Neg	Unknown	Yes
DC-1	HDC	Cadaveric kidney	Unknown	Neg	Unknown	Yes
M-RTEC	Mouse RTEc	Mouse kidney	NA	NA	NA	No

[a] HRTEc, human renal tubular epithelial cells; HRPTEc, human renal proximal tubular epithelial cells; HTALc, human thick ascending limb of Henle epithelial cells; HDC, human distal convoluted tubular epithelial cells
[b] m, months; Y, years; M, male; F, female; AA, African American; C, Caucasian
[c] HUS, hemolytic uremic syndrome; HIVAN, HIV associated nephropathy

(anti-V3/V4 domains; 10 μg/ml), or Leu 3a (Becton Dickinson, Mountain View, CA, USA; 10 μg/ml) for one hour. HIV-1 infected PBMCs were preincubated with these antibodies for one hour. RPTEc and PBMCs were co-cultured in medium containing 10 μg/ml of OKT4 or anti-Leu 3a antibodies for 12 to 24 hours. Infection was assessed as previously described. Controls included human RPTEc exposed to infected PBMCs in the absence of anti-CD4 monoclonal antibodies. The presence of CD4 protein in human RTEc was examined in cells fixed with 2% paraformaldehyde by fluorescein-activated cell scanning analysis, following standard procedures and using anti-Leu 3a monoclonal antibody (Becton Dickinson) and an isotype-matched irrelevant mouse monoclonal antibody as a control for non-specific staining.

Trans-filter infection experiments

To determine whether macrophages were able to infect RPTEc in the absence of cell fusion, RPTEc were seeded on permeable Nucleopore filters (3 μm) which were placed in Transwell cell culture chambers (Costar, Cambridge, MA, USA), above the macrophages. Macrophages, isolated from primary PBMCs by their ability to attach to the culture dishes, were infected with cell-free HIV-1 (DC-4). $RPTE_c$ seeded on the upper surface of five filters were placed in the Transwell cell culture chambers above the infected macrophages. Controls included human RPTEc derived from the same clone, seeded on two filters and exposed to uninfected macrophages, as well as non permissive MDCK cells seeded on three filters and exposed to HIV-1 infected macrophages. After 48 hours of exposure, the filters with the attached $RPTE_c$ were transferred to new culture dishes in the absence of macrophages, the culture media was changed, and the cells were followed for 10 to 14 days.

P24 antigen values were measured by an antigen capture enzyme immunoassay from Abbott Laboratory (North Chicago, IL, USA), following the manufacturer's directions. The upper detection limit of the assay was 200 pg/ml.

Detection of proviral DNA by polymerase chain reaction

Electrochemiluminescent detection of the HIV-1 specific amplification products were measured as described by Schutzbank and Smith [39]. Briefly, cells were removed and suspended in 250 μl of lysis buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.5 mM MgCl, 0.45% Nonidet P-40, 0.45% Tween 20, and 10 μg/ml proteinase K). The lysates were incubated at 60°C for one hour, followed by 100°C for 10 minutes to inactivate the proteinase K. A 50 μl sample of each specimen was transferred to a thin walled, 200 μl MicroAmp tube from Perkin Elmer (Norwalk, CT, USA) containing 50 μl of amplification master mix (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 500 μM each dNTP, 500 μM each SK38 and SK39-biotin primers and 2.5 units of AmpliTaq DNA polymerase (Perkin Elmer). The HIV-1 gag gene-specific PCR primers, SK38/5′ biotinylated-SK 39, and the detection probe SK 19, were synthesized by Genosys Biotechnologies Inc. (The Woodlands, TX, USA). Each PCR study was done in the presence of 3 negative controls (HIV-negative PBMCs) and two positive controls (10 and 50 copies of HIV-1 proviral DNA). In addition, control and infected human RTEc, as well as nonpermissive epithelial cells (MDCK or mouse RTEc) were run simultaneously in duplicates.

Detection of viral RNA by nucleic acid sequence base amplification

RNA was extracted from control and infected renal epithelial cells as described previously [11]. Quantification of viral genomic RNA was performed using a commercially available HIV-1 Q NASBA kit, from Organon Technica, (Research Triangle Park, NC, USA) following the manufacturer's directions.

Immunocytochemistry

For indirect immunofluoresence staining, cells were fixed in methanol for five minutes, washed three times in PBS and incubated for one hour at room temperature with HIV-positive human serum diluted 1:100 in PBS containing 3% BSA. After three washes in PBS, the coverslips were incubated for one hour at room temperature in rabbit anti-human fluorescein isothiocyanate (FITC)/green (Sigma) diluted in 1:80 in PBS with 3% BSA. Background and nonspecific staining were determined by using mock-infected cells. For HIV-1 p24 antigen staining we used a Kal-1 monoclonal antibody to HIV-1 p24 antigen from Dako (Glostrup, Denmark) and a peroxidase-avidin-biotin complex

(ABC) method from Dako [11]. All sections were counterstained with hematoxylin. A panel of different antibodies was used to identify the cell type infected by HIV-1; mouse monoclonal EBM-11 (1:4,000 dilution) and KP1 anti human CD68, which recognize monocyte/macrophages, anti-CD4, anti-CD8, leukocyte common antigen (LCA), cytokeratin, vimentin, (all from Dako) and α smooth muscle actin (Sigma). Goat anti-mouse IgG, as well as other second biotinylated antibodies, mouse PAP and mouse APAAP were obtained from Dako.

In situ hybridization

In situ hybridization studies were performed as previously described [40], using a digoxigenin-UTP labeled RNA probe from Lofstrands Labs, (Gaithersburg, MD, USA), generated by SP6 or T7 polymerase transcription from 1 to 2 kb subclones spanning the entire HIV-1 genome. Briefly, 4% paraformaldehyde fixed human primary macrophages and RTEc cell cultures were pretreated with 0.2 N HCl, proteinase K and hybridized with 1.75 ng/μl of sense or antisense HIV-1 riboprobes at 52°C overnight. Posthybridization treatment consisted of washing sequentially in 2 × SSC/50% formamide solution at 50°C, 1 × SSC and RNase solution at 37°C (RNase T1 and RNase A). The samples were blocked with 2% horse serum, 150 mM NaCl, and 100 mM Tris pH 7.4 for one hour, and stained with sheep anti-digoxigenin alkaline phosphate conjugate (Boehringer Mannheim, Germany) 1:500, and rinsed in Tris pH 7.4. The cells were then incubated with NBT/BCIOP substrate from Vector Labs, (Burlingame, CA, USA), containing 5 mM levamisole, overnight at room temperature. The stained slides were rinsed in distilled water counterstained with nuclear fast red, dehydrated and coverslipped. Controls for *in situ* included: HIV-1 sense riboprobe hybridized with HIV-1 infected cell cultures, and uninfected primary cell cultures hybridized with HIV-1 antisense probe.

Transmission electron microscopy

RTEc were washed one time with 1 × PBS and fixed in 3% glutaraldehyde in a 0.2 M phosphate buffer, pH 7.2 for detection of HIV-1. All experiments were performed in triplicate.

Changes in renal tubular epithelial cell morphology and growth

Renal tubular epithelial cells (RT-1) were seeded in six-well plates at a density of 5×10^5 cells in 0.5 ml of RTEc culture media. Cells on three dishes were exposed to HIV infected supernatants (0.5 ml/1 × 10^7 IU/ml), while the remaining control cells were exposed to supernatants from stimulated HIV-negative PBMCs. After five days of exposure, all cells were washed, trypsinized, counted, and passaged at equal cell densities (5×10^5 cells/well) into new tissue culture dishes in fresh RTEc culture media. Morphologic changes were evaluated daily under a light microscope. After one week, RTEc from both groups were counted. Results were expressed as the mean ± SD of values obtained in duplicate from at least two different experiments. Difference in cell number between control and HIV-1 treated groups were compared by Student's *t*-test. *P* values less than 0.05 were considered significant.

RESULTS

Infection of renal tubular epithelial cells derived from children with HIVAN

To examine whether RTEc derived from children with HIVAN were already infected with HIV-1, 35 clones were isolated from five different children and screened by PCR for the presence of HIV-1 proviral DNA. Two primary RTEc clones from two different patients were positive. The possibility that these colonies were contaminated by macrophages was excluded by (*1*) cloning and expanding colonies of RTEc in selective renal proximal tubular epithelial cell culture media; (*2*) performing immunohistochemistry studies with antibodies against cytokeratin, macrophages and lymphocytes (CD-68/LCA); (*3*) excluding the presence of contaminating cells by light microscopy and TEM in HIV-1 positive RTEc (Fig. 2).

To determine whether uninfected RTEc derived from children with HIVAN were susceptible to infection with their own primary HIV-1 isolates, RTEc were exposed to cell-associated and cell-free HIV-1 derived from peritoneal fluid or PBMCs of the same patients (Table 1). RTEc could be infected by their corresponding HIV-1 isolated from the same patient at a multiplicity of infection 1 and 10. Infection was accomplished using both HIV-1-infected PBMCs (Fig. 3) and cell-free viral supernatants (Fig. 4). Fourteen to twenty days after co-culture, supernatant p24 antigen levels ranged from 20 to 100 pg/ml (1×10^6 cells), which is 2 to 10 times greater than p24 levels from HIV-1 exposed non-permissive epithelial cells (Fig. 5). After 20 days in culture, HIV DNA PCR and HIV RNA NASBA values in infected RTEc were increased an average of 10 to 20 times the values of control human RTEc not exposed to HIV-1 or of non-permissive epithelial cells exposed to a similar number of infection units in both cell free and cell associated infection experiments (Figs. 3 and 4, legends). Human RTEc sustained low levels of productive infection, while mouse primary renal tubular epithelial cells or the nonpermissible renal canine epithelial cell line MDCK (Fig. 3) were not infected by either of the two primary isolates derived from the same patients (RW-2 and BS-3). These primary viral isolates also infected HeLa-CD4+ cells and peripheral blood-derived macrophages from HIV-1 negative donors, often inducing giant cell formation (Fig. 6C), and yielding p24 antigen levels in the macrophage culture media of above 100 pg/ml, as well as consistent HIV DNA PCR values $>$ 5,000 ECL units/1 × 10^6 cells and HIV-RNA NASBA values $>$ 20,000 copies/1 × 10^6 cells. In three experiments, infected human primary RTEc were detached and passaged onto collagen I coated dishes, but they subsequently lost their p24 antigen values, PCR-detectable DNA, and HIV-RNA (p24 $<$ 12.5 pg/ml; HIV-PCR DNA $<$ 1,000 ECL units; HIV-RNA $<$ 700 copies/per 1 × 10^6 cells, respectively) after three weeks in culture, suggesting that there can be a transient HIV-1 infection and/or that infected cells do not survive tissue culture passages.

Infection of renal tubular epithelial cells derived form HIV-1 negative patients

To ascertain whether RTEc from HIV-1 negative patients were also susceptible to infection, epithelial cells derived from proximal thick ascending limb of Henle, and distal convoluted tubules, were exposed to HIV-1. Two primary HIV-1 isolates from children with HIVAN, HIV-1 DC-4 and RW-2, were able to infect RTEc from HIV-negative children at MOIs ranging from 1 to 10. In all

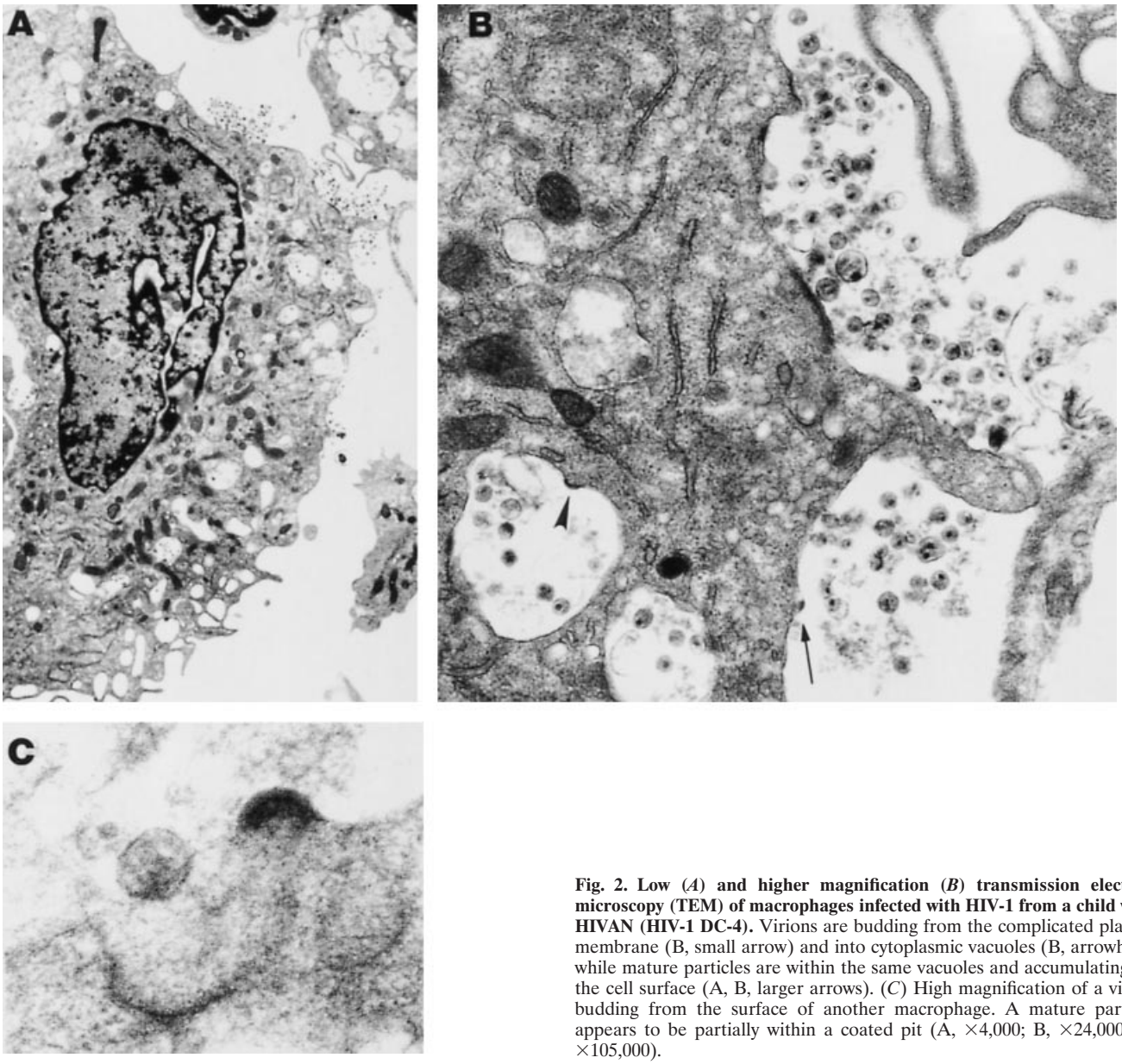


Fig. 2. Low (*A*) and higher magnification (*B*) transmission electron microscopy (TEM) of macrophages infected with HIV-1 from a child with HIVAN (HIV-1 DC-4). Virions are budding from the complicated plasma membrane (B, small arrow) and into cytoplasmic vacuoles (B, arrowhead while mature particles are within the same vacuoles and accumulating on the cell surface (A, B, larger arrows). (*C*) High magnification of a virion budding from the surface of another macrophage. A mature particle appears to be partially within a coated pit (A, ×4,000; B, ×24,000, C, ×105,000).

experiments nonpermissive MDCK cells and control human RTEc showed HIV DNA PCR values consistently < 800 ECL units/1 $\times$ 10^6 cells, and HIV RNA NASBA values < 700 copies/1 $\times$ 10^6 cells. Infected human RTEc showed consistent HIV-DNA PCR values $> 2{,}600$ ECL units/1 $\times$ 10^6 cells, and HIV RNA NASBA values $> 2{,}500$ copies/1 $\times$ 10^6 cells. In two different experiments human renal proximal tubular epithelial cells (RPTEc) were not infected by the T-lymphotropic III_B laboratory strain, while HeLa CD4+ cells subjected to a similar infection protocol were infected. Multiple PBMC passages of primary viral isolates from patients with HIVAN decreased their ability to infect human RTEc, since RPTEc exposed to HIV-1 RW-2 (which was passaged 5 times in PBMCs) showed p24 antigen levels < 12.5 pg/ml in the culture supernatants and viruses could not be rescued from these cells, after three weeks in culture.

Mechanisms of renal tubular epithelial cell infection

To investigate the role of the CD4 receptor during HIV-1 infection of RTEc, we attempted to block infection by using anti-CD4 antibodies. Infection of human RTEc was not inhibited by OKT4 or anti-Leu 3a monoclonal antibodies. In agreement with these results, we did not find CD4 receptors in cultured human RTEc by flow cytometry and Northern blot analysis of poly A^+ mRNA (data not shown). These findings suggest that HIV-1 enters RTEc by CD4 independent pathways.

Trans-filter experiments

To determine whether macrophages were able to infect renal proximal tubular epithelial cells (RPTEc) in the absence of cell fusion, RPTEc were cultured on permeable filters, which were

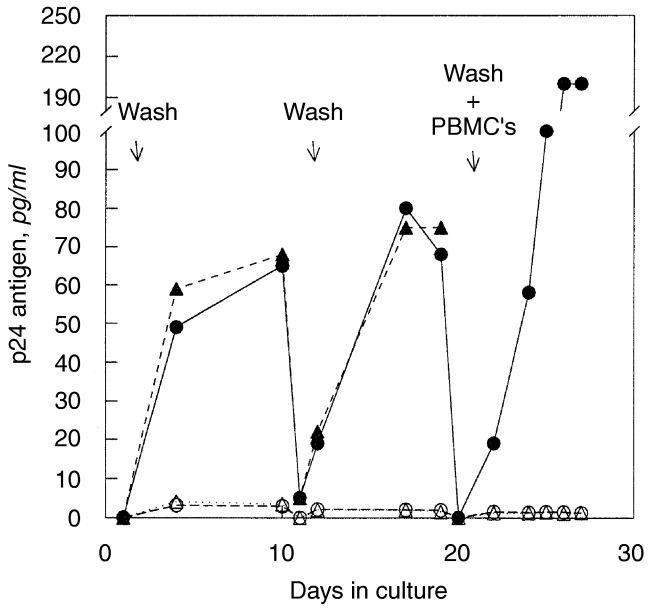


Fig. 3. Cell-to-cell associated infection of human primary renal tubular epithelial cells (RTEc). RTEc (1×10^6) derived from a child with HIVAN (RW) were exposed to 3×10^5 PBMC, infected with either HIV-RW-2, a viral isolate from the same patient (RW), or HIV-BS-3, a viral isolate corresponding to another child with HIVAN (BS). Cultured cells were followed for a period of three weeks as described in the **Methods** section. The arrows point to p24 antigen values measured immediately after 3 washes. Since the upper limit of detection of the p24 assay was 200 pg/ml, the scale was interrupted at this value. To eliminate the possibility that adherent viruses were being detected, experiments were controlled by exposing MDCK cells (a nonpermissible renal canine epithelial cell line (ATCC) or mouse primary RTEc (data not shown), to the same viruses. MDCK-1 or MDCK-2 exposed to HIV-1 RW-2 and HIV-BS-3 respectively, remained uninfected. On day 20, HIV-1 DNA PCR values in control samples were $<$ 800 ECL units/1×10^6 cells and HIV RNA nucleic acid sequence base amplification (NASBA) values $<$ 700 copies/1×10^6 cells. Infected cells showed mean HIV-1 DNA PCR values of 8,900 $\pm$ 1,110 ECL units/1×10^6 cells and HIV RNA NASBA values 16,000 $\pm$ 2,230 copies/1×10^6. Symbols are: (●) RTEc (RW)-HIV-1 (RW-2); (▲) RTEc (RW)-HIV-1 (BS-3); (○) MDCK1-HIV-1 (RW-2); (△) MDCK2-HIV-1 (BS-3).

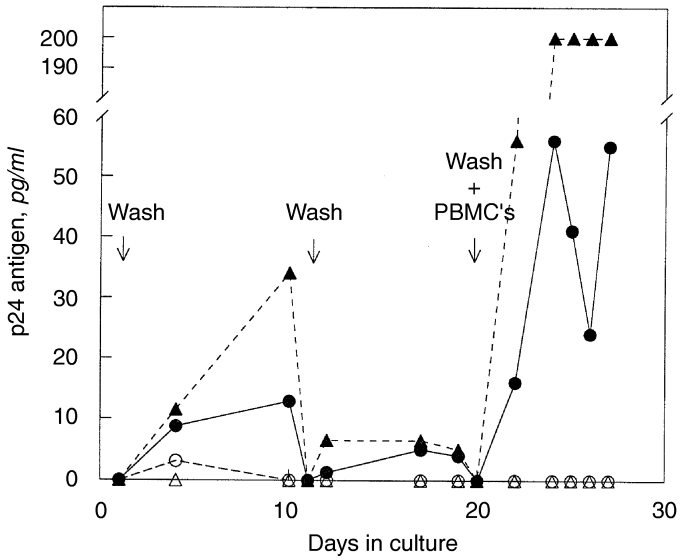


Fig. 4. Cell free infection of human primary renal tubular epithelial cells (RTEc). RTEc derived from a child with HIVAN (BS) were exposed to 0.5 ml of cell free virus ($>1 \times 10^7$ IU/ml) derived from peripheral blood mononuclear cell (PBMC) cultures infected with HIV-1 (BS-3; ●), a viral isolate from the same patient (BS), or HIV-1 (RW-2; ▲), the viral isolate corresponding to another child with HIVAN (RW). BS-RTEc, exposed to uninfected PBMC's supernatants were used as control cells [Mock-infected 1 (○) and Mock-infected 2 (△)]. RTEc were infected simultaneously with each particular stock of virus. After 24 to 48 hours exposure, cells were washed three times to remove the input virus and refed with fresh culture media and followed for a period of three weeks as described in the **Methods** section. The amount of HIV-1 p24 antigen production was measured every two-three days by ELISA. The arrows point to p24 antigen values measured immediately after three washes. Since the upper limit of detection of the p24 assay was 200 pg/ml, the scale was interrupted at this value. Experiments were terminated by testing the ability of RTEc to infect normal HIV-1 negative PBMC. All infected RTEC were able to infect co-cultured PBMC, while uninfected RTEc did not. On day 20 HIV-1 DNA PCR values in control samples were $<$ 800 ECL units/1×10^6 cells and HIV RNA NASBA values $<$ 700 copies/1×10^6 cells. Infected cells showed mean HIV-1 DNA PCR values of 2,300 $\pm$ 570 ECL units/1×10^6 cells, and mean HIV RNA NASBA values of 6,400 $\pm$ 1,008 copies/1×10^6 cells.

then placed in culture chambers allowing independent access to either the apical or basal surfaces. HIV-1 infected macrophages were cultured in the lower chamber, beneath the uninfected RPTEc that were seeded on the upper surface of the filters. After 48 hours of incubation, the filters with the attached RPTEc were transferred to fresh dishes and cultured for 10 to 14 days. Three of five human RPTEc cultures became infected with HIV-1, while none of three control MDCK cell cultures was infected based on the same infection criteria described in the **Methods** section.

Transmission electron microscopy studies

Productively-infected lymphocytes and macrophages came into intimate contact with the surface of epithelial cells (Figs. 7 and 8), and virions were detected in the intervening space and within cytoplasmic vacuoles of macrophages and RTEc (Fig. 7, A-C). Infection of renal epithelium was more effective in the presence of intimate contact of mononuclear cells and RTEc.

Growth of renal tubular epithelial cells exposed to HIV-1

Renal tubular epithelial cells exposed to HIV-1 infected supernatants showed significant evidence of injury and decreased growth rates. Under light microscopy (Fig. 9), epithelial cell injury was manifested by the loss of cell to cell contacts, acquisition of spindle morphology, decreased growth rates, increased cell detachment from the culture dish, and eventually necrosis. Injury was confirmed by TEM and apparently not limited to infected cells, suggesting that other factors produced by HIV-1 infected PBMCs may have a role in this process. Two different experiments using different primary viral isolates (DC-4 and RW-2) showed a significant reduction in the final number of cells exposed to HIV-1 ($3.6 \pm 0.3 \times 10^6$ cells/well in control, vs. $2.1 \pm 0.2 \times 10^6$ cells/well in HIV-1 exposed cells ($P < 0.001$).

DISCUSSION

Distinctive histologic features of HIVAN are the swelling of visceral glomerular epithelial cells, atrophy of tubular epithelium,

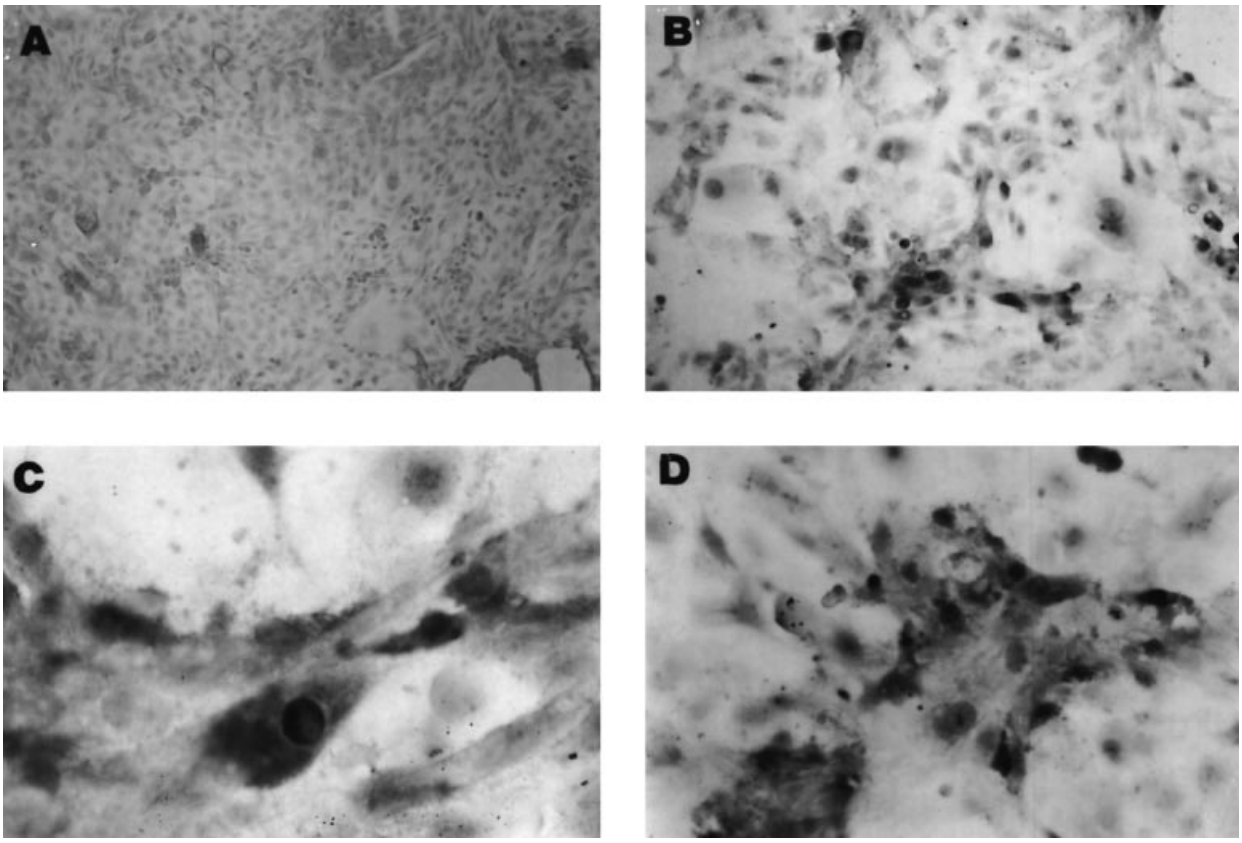


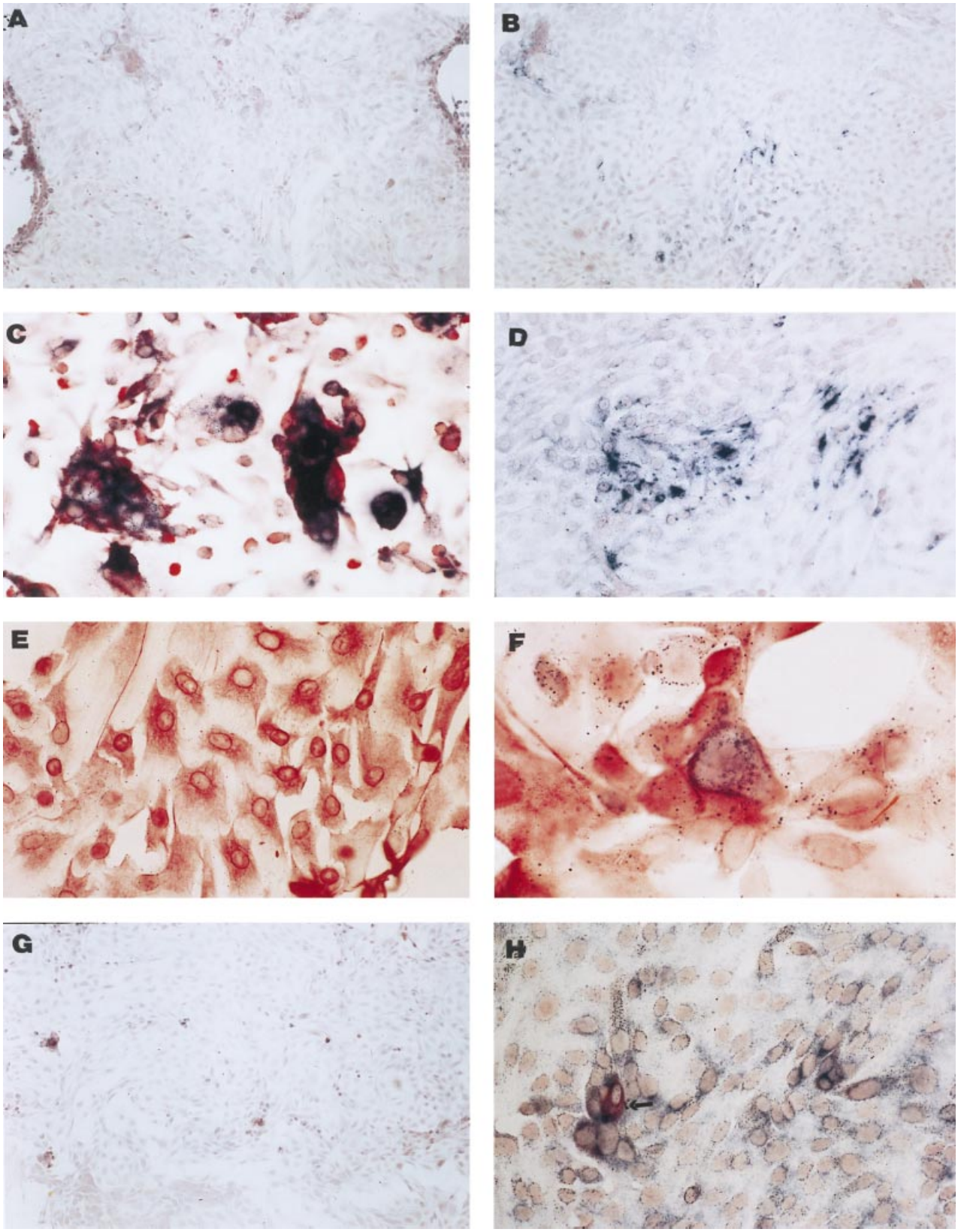
Fig. 5. Immunostaining of primary human renal tubular epithelial cells (RTEc) infected with HIV-1 isolates derived from children with HIVAN in cell associated infection experiments. (*B, C and D*) demonstrate HIV-1 p24 antigen staining detected in the cytoplasm of human RTEc using a Kal-1 monoclonal antibody to HIV-1 p24 antigen (Dako) (B ×200, C ×630, D ×400) and a peroxidase-avidin-biotin complex (ABC). (*A*) Uninfected RTEc exposed to the same antibody (×100). All sections were counterstained with hematoxylin.

and microcystic tubular dilation. An important question remaining to be answered, is whether HIV-1 can productively infect and injure renal epithelial cells. In the present study, we isolated primary renal tubular epithelial cells from children with HIVAN and exposed them to the viral isolates derived from the same patients. Our results provide a strong evidence to support the hypothesis that HIV-1 is capable of infecting and injuring renal tubular epithelial cells.

HIV-1 can enter epithelial cells by three possible routes: binding of cell-free virus to specific receptors, fusion with infected mononuclear cells, and direct transfer during intimate contact between productively-infected mononuclear cells and epithelial cells [24, 25]. In the last two instances, the virus is isolated from the immune system of the host. In agreement with the third mechanism, Phillips and co-workers [25, 26] showed that HIV-1 infected mononuclear cells interact with specific binding sites on cervical epithelial cells (MT180), and release viruses into the intervening space. We found that all primary viral isolates capable of infecting RTEc could infect macrophages. Productively-infected lymphocytes and macrophages came into intimate contact with the surface of epithelial cells and virions were detected in the intervening space and within cytoplasmic vacuoles of macrophages and renal epithelial cells. Thus, considering the high viral titers needed to infect cultured RTEc, the renal epithelium *in vivo* may be more likely to be infected from intimate contact of mononuclear cells and RTEc.

The mechanism(s) responsible for HIV-1 infection of RTEc is not completely understood at the present time. Green, Resnick and Bourgoignie were unable to infect cultured renal glomerular epithelial cells from one patient using a low passage clinical strain of HIV-1 isolated from a patient with AIDS and the T-lymphotropic III_B laboratory strain of HIV-1 [16]. We could not infect RPTEc by using the T-lymphotropic laboratory strain of HIV-1 III_B. Results from both studies, however, are difficult to compare, since in addition to different renal epithelial cell type, we have used primary isolates and RTEc derived from children with renal

Fig. 6. *In situ* hybridization studies performed on primary macrophages and RTEc derived from children with HIVAN and infected with their corresponding primary isolates in cell associated infection experiments. (*A*) Infected renal tubular epithelial cells (RTEc) hybridized with HIV-1 sense probe (×100). (*B and D*) Different magnifications of infected RTEc hybridized with HIV-1 antisense probe. Positive HIV-1 RNA was detected within the cytoplasm of some RTEc (B ×100 and D ×360). (*C*) Colocalization of HIV-1 RNA (blue) by *in situ* hybridization in cultured macrophages, which are stained red by immunohistochemistry using an antibody against CD-68 antigen (C ×360). (*E*) Cytokeratin expression in renal tubular epithelial cells



by immunohistochemistry (×500). (*F*) Colocalization of HIV-1 RNA (blue) and cytokeratin (red) within the cytoplasm of a RTEc stained by ISH and IHC (F ×800). (*G*) Control human RTEc hybridized with HIV-1 antisense probe (G ×100). (*H*) A co-localization study of HIV-RNA by *in situ* hybridization (blue) and mononuclear cells (red) by immunohistochemistry, using an anti-CD-68 antibody which stains mononuclear cells (arrow). Note HIV-RNA cytoplasmic staining in human RTEc that are not stained (H ×360).

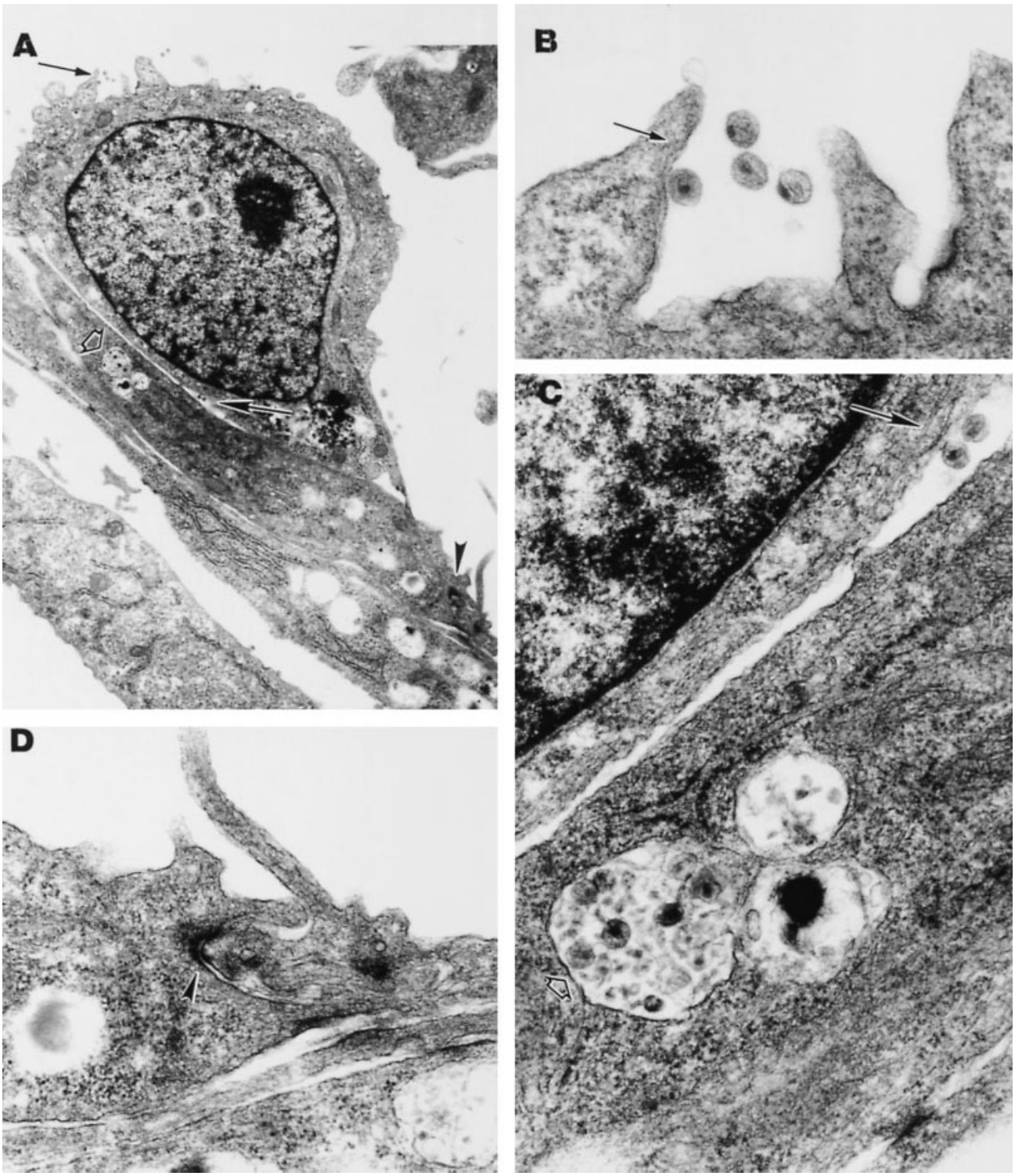


Fig. 7. Macrophage spread over portion of surface of flattened renal tubular epithelial cells (RTEc) (*A*). Mature HIV-1 particles are associated with surface of macrophage (A, small arrow, upper left field and *B*) including in the narrow intercellular space (A, larger arrow, center of field and in *C*). Several mature particles are within cytoplasmic vacuole of RPTEc (A & C open arrows). A desmosome joins lateral surface of 2 epithelial cells (A & D solid arrowheads) (magnification: A ×7,000, B ×58,000, C ×58,000, D ×33,000).

disease, higher infection units, and different experimental design. It is possible, however, that viruses from children with HIVAN may have adapted to infect RTEc and that this capacity is lost during passage through PBMCs. Moreover, infection of RTEc was not significantly inhibited by anti-CD4 antibodies, suggesting that this process is mediated by CD4 independent pathways. In

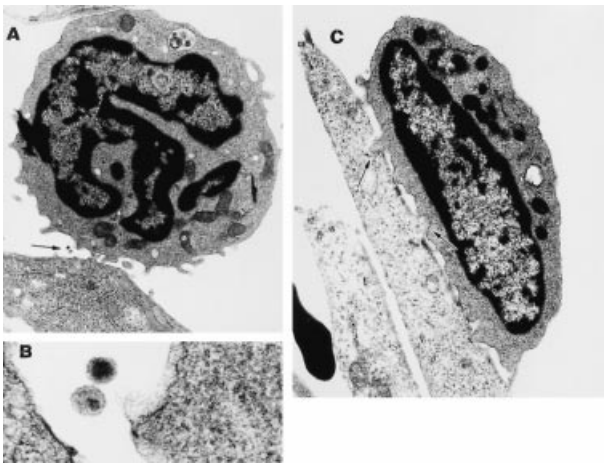


Fig. 8. Two mature virions are located in the intervening space between a spherical lymophocyte and a flattened renal proximal tubular epithelial cell RPTEc (*A*, arrow, and *B*). The flattened intimately-associated lymphocyte and RPTEc appear to have interdigitating process (*C*, arrows) (magnification: A ×12,000, B ×105,000, C ×16,000).

agreement with this result, we did not find CD4 receptors in cultured RTEc. More studies will be required to identify the receptors involved in this process. For example, the sulfate chains of heparan sulfate proteoglycans (HSPG), a known facilitator of virus adsorption [41, 42], other newly described co-receptors [30–34], or adhesion molecules [29] may play a relevant role in this process. Thus, HIV-1 cell tropism [43, 44], in addition to cellular factors present in human renal epithelial cells, can influence infection. Finally, we have demonstrated that RTEc isolated from both African-American and Caucasian children can be infected with HIV-1 isolates derived from children with HIVAN. This finding suggests that other co-factors and/or genetic predisposition of black patients, in addition to HIV-1 infection of RTEc, may play an important role in the pathogenesis of HIVAN.

Renal tubular epithelium may be infected through basolateral or luminal surfaces. Though HIV-1-infected cells and free virus have been detected in the urine of patients [45], it is difficult to culture HIV-1 from the urine or to infect RTEc with concentrated urine samples. This evidence suggests that RTEc are more likely to be infected through their basolateral surfaces by tissue mononuclear cells. We found that RTEc cultured on permeable filters and placed in culture chambers, which allow an independent access to either the apical or basal surfaces, were infected by macrophages through their basolateral surfaces and without cell-to-cell fusion. This may be the most likely *in vivo* mechanism of RTEc infection in children.

A major issue in the pathogenesis of HIVAN is whether RTEc can actually replicate HIV-1. Transmission electron microscopy confirmed the presence of mature virions within cytoplasmic vacuoles in RTEc. It is possible that some viral particles may undergo lysosomal digestion, but it is unlikely that all our results could be accounted for solely on the basis of this process. The detection of HIV RNA by NASBA and *in situ* hybridization in RTEc, as well as the detection of proviral DNA and cytoplasmic p24 antigen, strongly suggest that viral-specific RNA and protein are being synthesized and that RTEc are productively infected. That PBMCs cocultured with cell-free infected RTEc themselves become productively infected also support this contention. Our failure to rescue HIV-1 viruses from non permissive epithelial cells or HIV-1 III_B from the cell surface of human RTEc demonstrates that under the current experimental conditions adherent viruses do not survive more than three weeks in culture. Moreover, studies in transgenic mice demonstrate the presence of all factors necessary for HIV -1 gene expression in RTEc [9–11]. However, the high viral titers used to infect cultured RTEc, in addition to the low levels of p24 antigen produced by infected RTEc, and the difficulties in detecting viral antigens in kidney sections from patients with HIVAN, suggest that caution is necessary before extrapolating these results to the *in vivo* situation. The low yield of infected urinary RTEc derived from the urine of children with HIVAN also suggests that other factors, in addition to the infection of RTEc, may play a relevant pathogenic role in HIVAN. It is possible, however, that current *in situ* hybridization techniques may not be sensitive enough to detect low level replication of HIV in renal cells *in vivo.* Alternatively, productively infected RTEc may succumb to viral infection and/or become latently infected. This hypothesis may also explain our difficulties in culturing and expanding infected primary RTEc from the urine of children with HIVAN or RTEc infected *in vitro*. The high number of regenerating RTEc present in renal sections from children with HIVAN [46] suggests that infected cells may be rapidly replaced.

An additional relevant finding of this study was the induction of epithelial cell injury by HIV-1. The magnitude of the cell injury depended on tissue culture conditions, degree of cell confluency, type of renal tubular epithelium, and extracellular matrix used to coat the culture dishes. Subconfluent cells, cells grown directly on plastic, and human primary RTEc derived from non-infected patients, were more sensitive to HIV-1 exposure than confluent cells, cells grown on collagen coated dishes, or cells isolated from HIV-1 infected patients. The mechanism(s) responsible for HIV-induced injury of RTEc is currently under investigation. Mice made transgenic with a replication defective subgenomic proviral HIV-1 construct, manifest renal tubular epithelial cell injury in correlation with the induction of high levels of HIV gene expression [9–11, 47]. In addition, cultured transgenic RTEc expressing high levels of HIV genes grow slower than RTEc isolated from their control littermates (Ray, unpublished data). Thus, HIV-1 infection or HIV-1 gene products, may have a direct growth inhibitory effect in RTEc independent of other environmental factors.

In summary, we have demonstrated that primary human RTEc can be infected with primary HIV-1 isolates derived from children with HIVAN through a CD4 independent pathway. Tissue mononuclear cells may play an important role in this process and can transfer the virus to epithelial cells. Moreover, infection and/or expression of HIV-1 gene products inhibited the *in vitro* growth and survival of primary RTEc. These findings suggest that the renal epithelial degenerative changes present in children with HIVAN may, at least in part, be explained by the infection of RTEc by HIV-1.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health Grants R0-1 DK49419 and R0-1 HL55605 and by "F.A.D.I." from Buenos Aires, Argentina. We thank Dr. P.D. Wilson for providing primary cultures of renal proximal, thick ascending limb and distal convoluted tubular cells;

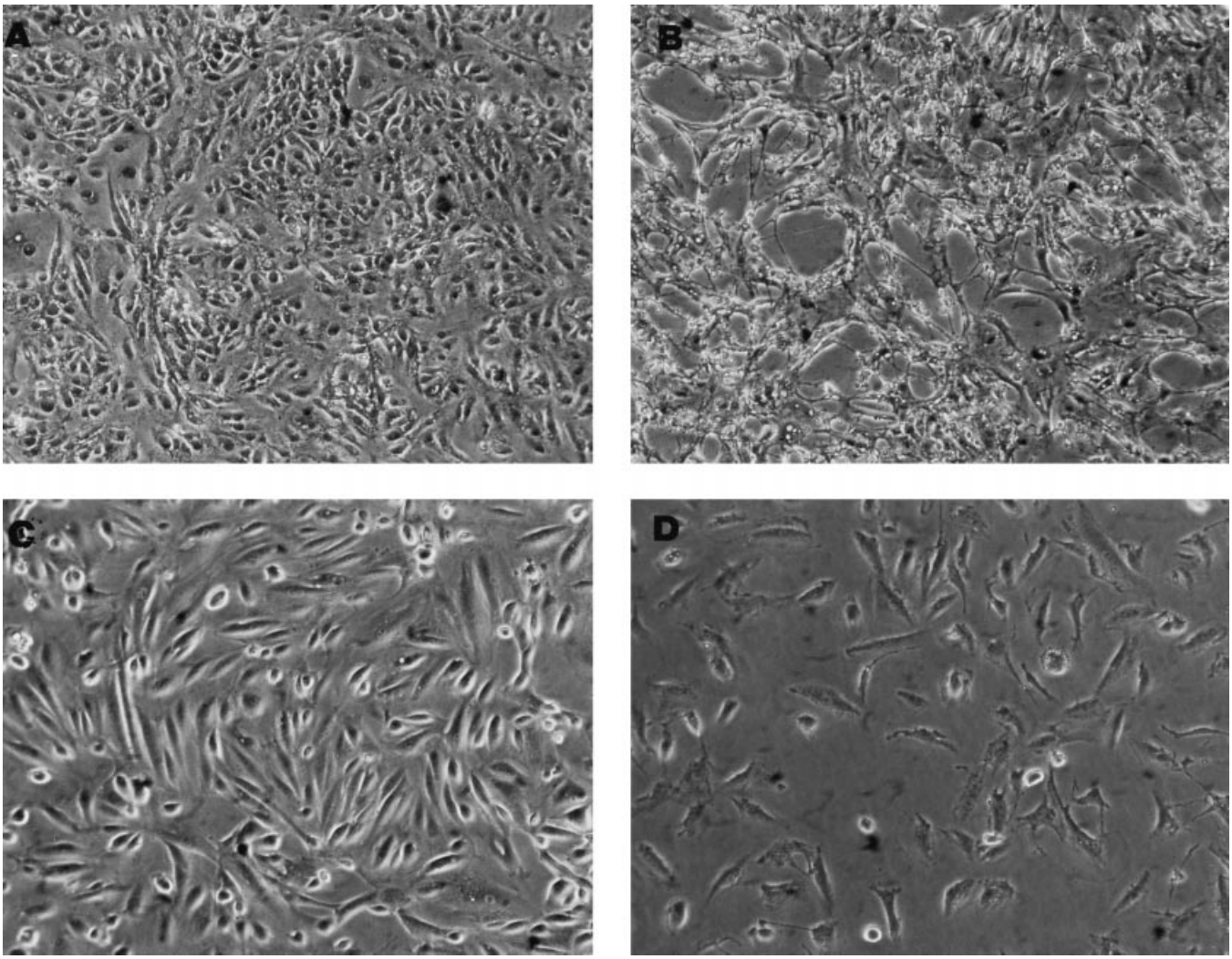


Fig. 9. Effects of HIV-1 on cell growth. (*A*) Human renal tubular epithelial cells (RTEc; RT-1) exposed for five days to control supernatants derived from stimulated HIV-negative peripheral blood mononuclear cells (PBMCs). (*B*) Human RTEc (RT-1) exposed for five days to HIV-infected supernatants as described in the **Methods** section. After five days, all cells in both groups were washed, trypsinized, counted, and passaged at equal cell densities into new culture dishes. (*C*) Control RTEc cells growing in normal RTEc culture media, five days after the second passage. (*D*) RTEc cells previously exposed to HIV-1, growing in normal RTEc culture media five days after the second passage. Note cell detachment, changes in cell morphology, and slower growth rate of RTEc exposed to HIV-1 (B and D) (magnifications: A and B ×100; C and D ×200).

Dr. C.R. Brown for assistance in performing *in situ* hybridization studies; J. Smith, V. Gopal and S. Ellis for technical assistance; Barbara Taylor for performing flow cytometry studies; Seth Honig, Dr. Inke Sunila for EM assistance.

Reprint requests to Patricio E. Ray, M.D., Children's Research Institute, Research Center IV, Room R-211, Children's National Medical Center, 111 Michigan Ave. N.W., Washington, D.C. 20010, USA.
E-mail: Pray@cnmc.org

REFERENCES

1. Rao TK, Filippone EJ, Nicastri AD, Landesman SH, Frank S, Chen CK, Friedman EA: Associated focal and segmental glomerulosclerosis in the acquired immunodeficiency syndrome. *N Engl J Med* 310:669–673, 1984
2. Pardo V, Aldana M, Colton RM, Fischl MA, Jaffe D, Moskowitz L, Hensley T, Bourgoignie JJ: Glomerular lesions in the acquired immunodeficiency syndrome. *Ann Intern Med* 101:429–434, 1984
3. Gardenswartz MH, Lerner CW, Seligson GR, Zabetakis PM, Rotterdam H, Tapper ML, Michelis MF, Bruno MS: Renal disease in patients with AIDS: A clinicopathological study. *Clin Nephrol* 21:197–204, 1984
4. Strauss JC, Abitol C, Zilleruelo G, Scott G, Paredes A, Malaga S, Montane B, Mitchell C, Parks W, Pardo V: Renal disease in children with acquired immunodeficiency syndrome. *N Engl J Med* 321:625–630, 1991
5. Humphreys MH: Human immunodeficiency virus-associated glomerulosclerosis. *Kidney Int* 48:311–320, 1995
6. Cantor ES, Kimmel PL, Bosch JP: Impact of race on renal disease in patients with AIDS. *Arch Intern Med* 151:125–128, 1991
7. Bourgoignie JJ, Ortiz-Interian C, Green DF, Roth D: Race, a co-factor in HIV-1 associated nephropathy. *Transplant Proc* 21:3899–3901, 1989
8. D'Agati V, Suh JI, Carbone L, Cheng JT, Appel G: Pathology of HIV-associated nephropathy: A detailed morphologic and comparative study. *Kidney Int* 35:1358–1370, 1989
9. Dickie P, Felser J, Eckhaus M, Bryant J, Silver J, Marinos N, Notkins AL: HIV-associated nephropathy in transgenic mice expressing HIV-I genes. *Virology* 185:109–119, 1991

10. Kopp JB, Klotman ME, Adler SH, Bruggeman LA, Dickie P, Marinos NJ, Eckhaus M, Bryant JL, Notkins AL, Klotman PE: Progressive glomerulosclerosis and enhanced renal accumulation of basement membrane components in mice transgenic for human immunodeficiency virus type 1 genes. *Proc Natl Acad Sci USA* 89:1577–1581, 1992
11. Ray PE, Bruggeman LA, Weeks BS, Kopp JB, Bryant JL, Owens JW, Notkins AL, Klotman PE: Role of bFGF and its low affinity receptors in the pathogenesis of HIV-associated nephropathy in transgenic mice. *Kidney Int* 46:759–772, 1994
12. Cohen AH, Sun NCJ, Shapshak P, Imagawa DT: Demonstration of human immunodeficiency virus in renal epithelium in HIV-associated nephropathy. *Mod Pathol* 2:125–128, 1989
13. Nadasdy T, Hanson-Painton O, Davis LD, Miller KW, Debault LE, Burns DK, Silva FG: Conditions affecting the immunohistochemical detection of HIV in fixed and embedded renal and nonrenal tissues. *Mod Pathol* 5:283–291, 1992
14. Kimmel PL, Ferreira-Centeno A, Farkas-Szallasi T, Abraham AA, Garret CT: Viral DNA in microdissected renal biopsy tissue from HIV infected patients with nephrotic syndrome. *Kidney Int* 43:1347–1352, 1993
15. Dibelgiojoso GB, Genderini A, Vago L, Parravicini C, Bertoli S, Landriani N: Absence of HIV antiges in renal tissue from patients with HIV-associated nephropathy. *Nephrol Dial Transplant* 5:489–492, 1990
16. Green DF, Resnick L, Bourgoignie JJ: HIV infects glomerular endothelial and mesangial but not epithelial cells in vitro. *Kidney Int* 41:956–960, 1992
17. Lusso P, Lori F, Gallo RC: CD4 independent infection of human immunodeficiency virus type 1 after phenotypic mixing with human T-cell leukemia viruses. *J Virol* 64:6341–6344, 1990
18. Tateno M, Gonzalez-Scarano F, Levy JA: Human immunodeficiency virus can infect CD-4 negative human fibroblastoid cells. *Proc Natl Acad Sci USA* 86:4287–4290, 1989
19. Harouse JM, Kunsch C, Hartle HT, Laughlin MA, Hoxie JA, Wigdahl B, Gonzalez-Scarano F: CD4-independent infection of human neural cells by immunodeficiency virus type 1. *J Virol* 63:2527–2533, 1989
20. Li XL, Moudgil T, Vinters HV, Ho DD: CD4-independent, productive infection of a neuronal cell line by human immunodeficiency virus type 1. *J Virol* 64:1383–1387, 1990
21. Adachi A, Koenig S, Gendelman HE, Daugherty D, Gattoni-Celli S, Fauci AS, Martin MA: Productive, persistent infection of human colorectal cell lines with human immunodeficiency virus. *J Virol* 61:209–213, 1987
22. Fantini J, Yahi N, Chermann J-C: Human immunodeficiency virus can infect the apical and basolateral surfaces of human colonic epithelial cells. *Proc Natl Acad Sci USA* 88:9297–9301, 1991
23. Douglas GC, Fry GN, Thirkill T, Holmes E, Hakim H, Jennings M, King BF: Cell-mediated infection of human placental trophoblasts with HIV in vitro. *AIDS Res Hum Retroviruses* 7:735–740, 1989
24. Phillips DM, Bourinbaiar AS: Mechanisms of HIV spread from lymphocytes to epithelia. *Virology* 186:261–273, 1989
25. Bourinbaiar AS, Phillips DM: Transmission of human immunodeficiency virus from monocytes to epithelia. *J AIDS* 4:56–63, 1991
26. Pearce-Pratt R, Malamud D, Phillips DM: Role of cytoskeleton in cell-to-cell transmmission of human immunodeficiencey virus. *J Virol* 68:2898–2905, 1994
27. Hosmy J, Meyer M, Tateno M, Clarkson S, Levy JA: The Fc and not the CD4 receptor mediates antibody enhacement of HIV infection in human cells. *Science* 244:1357–1360, 1989
28. Harouse JM, Bhat S, Spitalnik SL, Laughlin M, Stefano K, Silberberg DHF, Gonzalez-Scarano F: Inhibition of entry of HIV-1 in neural cell line by antibodies against galactosyl ceramide. *Science* 253:320–322, 1991
29. Lee MM, Hildreth JEK: HIV acquires functional adhesion receptors from host cells. *AIDS Res Hum Retroviruses* 11:1007–1013, 1995
30. Feng Y, Broder CC, Kennedy PE, Berger EA: HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane G protein-coupled receptor. *Science* 272:872–876, 1996
31. Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, Berger EA: CC CKR5: A RANTES, MIP-1α, MIP 1β receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 272:1955–1958, 1996
32. Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, Wu L, MacKay CR, Larosa G, Newman W, Gerald N, Gerard C, Sodroski J: The β-chemokine receptors CCR3 and CCR5 faciliate infection by primary HIV-1 isolates. *Cell* 85:1135–1148, 1996
33. Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, Cayanan C, Maddon PJ, Koup RA, Moore JP, Paxton WA: HIV-1 entry into CD4+ cells is mediated by a chemokine receptor CC-CKR-5. *Nature* 381:667–673, 1996
34. Wilson PD: Monolayer of microdissected renal tubule epithelial segments. *J Tiss Cult Meth* 13:137–141, 1991
35. Chesebro B, Wehrly K, Metacalf J, Griffin DE: Use of a new CD4-positive HeLa cell clone for direct quantification of infectious human immunodeficiency virus from blood cells of AIDS patients. *J Infect Dis* 163:64–70, 1991
36. Chesebro B, Wehrly K: Development of a sensitive quantitative focal assay for human immunodeficiency virus infectivity. *J Virol* 62:3779–3788, 1988
37. Popovic M, Sarngadharan MG, Read E, Gallo RC: Detection, isolation and continuous production of cytopathic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 224:497–500, 1984
38. Pise CA, Newburger PE, Holland CA: Human immunodeficiency virus type 1-infected HL-60 cells are capable of both monocytic and granulocytic differentiation. *J Gen Virol* 73:3257–3261, 1992
39. Schutzbank TE, Smith J: Detection of Human immunodeficiency virus type 1 proviral DNA by PCR using and electrochemiluminescence-tagged probe. *J Clin Microbiol* 33:2036–2041, 1995
40. Hirsch VM, Dapolito G, Johnson PE, Elkins WR, London WT, Goldstein S, Montali R, Brown CR: Induction of AIDS by simian immunodeficiency virus from an African green monkey: Species-specific variation in pathogenicity correlates with extent of in vivo replication. *J Virol* 69:955–967, 1994
41. Patel M, Yanagishita M, Roderiquez G, Bou-Habib DC, Oravecz T, Hascall VC, Norcross MA: Cell-surface heparan sulfate proteoglycan mediates HIV-1 infection of T-cell lines. *AIDS Res Hum Retrovirus* 9:167–174, 1993
42. Roderiquez G, Oravecz T, Yanagishita M, Bou-Habib DC, Mostowski H, Norcross MA: Mediation of human immunodeficiency virus type 1 binding by interaction of cell surface heparan sulfate proteoglycans with the V3 region of envelop gp120-gp41. *J Virol* 69:2233–2239, 1995
43. Cheng-Mayer C, Seto D, Tateno M, Levy JA: Biologic features of HIV that correlates with virulence in the host. *Science* 240:80–82, 1988
44. Zhang LQ, MacKenzie P, Cleland A, Holmes EC, Leigh Brown AJ, Simmonds P: Selection for specific sequences in the external envelope protein of human immunodeficiency virus type 1 upon primary infection. *J Virol* 67:3345–3356, 1993
45. Li JJ, Huang YO, Poiesz BJ, Zaumetzger-Abbot L, Friedman-Kien AE: Detection of human immunodeficiency virus type 1 (HIV-1) in urine cell pellets from HIV-1 seropositive individuals. *J Clin Microbiol* 30:1051–1055, 1992
46. Liu X-H, Ray PE: Recruitment of epithelial cells expressing mesenchymal features in children with HIV associated nephropathy. (abstract) *J Am Soc Nephrol* 7:A2461, 1996
47. Bruggeman LA, Dikman S, Meng C, Quaggin SE, Coffman TM, Klotman PE: Nephropathy in human immunodeficiency virus-1 transgenic mice is due to renal transgene expression. *J Clin Invest* 100:84–92, 1997