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Adherence of human immunodeficiency virus-infected lymphocytes to fetal placental cells: A model of maternal → fetal transmission

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ABSTRACT **The precise timing and mechanism of *in utero* human immunodeficiency virus (HIV) infection are unknown, but transplacental transmission is likely. Term placentas from HIV$^+$ pregnancies contain only rare HIV-infected cells whose origins and phenotypes remain controversial, and no correlation has been found between the presence of HIV in term placentas and transmission to offspring. Reports of trophoblast infectibility have not been reproducible and do not address the question of infection in the placental stroma, the cells in direct contact with fetal circulation. We report that primary cultures of fetal placental chorionic villus stromal cells, while not infectable *in vitro*, do support lethally irradiated HIV-infected peripheral blood mononuclear cells (PBMCs) in a form that permits rescue of HIV by activated PBMCs weeks later. Infected PBMCs adhere and become intimately associated with placental cells by a mechanism that is LFA-1 and CD4 independent but can be blocked by antibodies or soluble CD4 binding to cell surface-expressed HIV envelope. The ability to sustain infected irradiated cells was not shared by several trophoblast, fibroblast, or epithelial cell lines. This model has several features that are compatible with *in utero* transmission and allow testing of various agents proposed as interventions to block maternal → fetal transmission. Placental stromal cells appear to inhibit apoptosis of HIV-infected, irradiated lymphocytes.**

Up to half of congenitally infected infants may acquire human immunodeficiency virus (HIV) *in utero*. Several studies have identified HIV antigens and/or proviral DNA in a high percentage of electively aborted first- and second-trimester fetuses from seropositive pregnancies (1–4). Miles *et al.* (5) demonstrated p24 antigen detected by acid dissociation in the umbilical cord blood of 5 out of 8 infected children and only 2 out of 22 uninfected children. Since there was no correlation between maternal and cord blood p24 levels, this was interpreted as reflecting prenatal infection.

Attempts to diagnose placental infection at time of delivery have been negative or nonpredictive of transmission (6, 7). Such studies are hindered by the differentiation and involution of the placenta throughout gestation, the impossibility of examining more than a fraction of the entire placenta, and contamination with maternal blood. Potentially infectable CD4$^+$ macrophage-like Hofbauer cells have been identified in term and first-trimester placentas (2, 8–13). While Hofbauer cells are a potential target and source of maternal → fetal HIV infection, some investigators have also reported the presence *in situ* of HIV provirus or antigen in nonmacrophage CD4$^+$ and CD4$^-$ cells (2, 14, 15). Others have failed to repeat these findings (6–8). Similarly, reports of cell-free HIV *in vitro* infection of chorionic villus micro-organ explants (16, 17, 37) or transformed trophoblast lines (18) have been controverted by more recent studies showing resistance to infection by cell-free virions (13) but apparent susceptibility to infection by cell-associated HIV of the same strain (19). In addition to the drawbacks inherent in studying transformed cells, studies with trophoblast tumor lines do not shed light on infection of the fetal placental stromal cell layer, which lies in direct contact with the fetal vasculature during gestation. In light of these problems, we have developed an *in vitro* model employing primary placental cells from uninfected 10- to 12-week fetuses.

MATERIALS AND METHODS

Cell Lines. HeLa and HeLa CD4$^+$ clone 6C (HT 6C) were obtained from the AIDS Research and Reference Reagent Program (Rockville, MD). JAR (trophoblast), CCD-18, and FHs 738 (fibroblast) lines were purchased from the American Type Culture Collection.

Isolation and Culture of Placental Stromal Cells. First-trimester (10–12 week) chorionic villus biopsy samples from HIV$^-$ subjects, obtained for metaphase chromosome analysis, were used as a source of placental stromal cells. Primary cultures were established from microscopically dissected villi digested with trypsin and collagenase (20). Resultant cell suspensions were cultured in Chang medium (Irvine Scientific) with 5% (vol/vol) fetal calf serum for the first week and maintained in αMEM (GIBCO) supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, penicillin (200 units/ml), and streptomycin (200 μg/ml).

Phenotypic Characterization. *FACS analysis.* Purified placental stromal cells growing as an adherent monolayer were incubated with 1% human IgG in PBS for 1 h at 37°C, washed twice, removed from flasks using cell dissociation buffer (Sigma), and labeled with fluorescein isothiocyanate-conjugated monoclonal F(ab')$_2$ anti-CD4 (Zymed) and anti-CD14 (Zymed).

Esterase staining. Cells grown on slide flasks (Nunc) for 3 days were assayed for esterase activity *in situ* by modified α-naphtholbutyrate esterase assay (21).

Immunohistochemistry. Cells grown in 8-well chamber slides for 3 days and fixed with acetone or formalin were stained with OKT4 (AMAC, Westbrook, ME), Leu-M1 (Beckton Dickinson), anti-vimentin (ICN), Kp1 (Dako), Mac 387 (Dako), OKM1 (hybridoma from the American Type Culture Collection), anti-human placental lactogen (Dako), and polyclonal anti-human chorionic gonadotropin (Dako). Binding of biotinylated anti-mouse IgG second antibody was developed with

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Abbreviations: HIV, human immunodeficiency virus; PHA, phytohemagglutinin; PBMC, peripheral blood mononuclear cell; PHA blast, PHA-stimulated PBMC; sCD4, soluble recombinant CD4; Ab, antibody; C, complement; mAb, monoclonal antibody; AZT, 3'-azido-3'-deoxythymidine; IU, infectious unit.

horseradish peroxidase streptavidin D, and hematoxylin was used as counterstain.

Immunofluorescence microscopy. Placental cells cultured for 1 week were washed with PBS and fixed with cold acetone/methanol (1:1, vol/vol) for 20 min. The cells were washed with PBS, incubated in 1% human IgG for 1 h at 37°C, and then stained with anti-CD4, anti-CD14, and anti-CD18 (Dako) in PBS/1% human IgG for 45 min. Cells were then washed five times with PBS and incubated with fluorescein isothiocyanate-conjugated anti-mouse IgG for 45 min, washed five times, and mounted.

Cell-Free and Cell-Associated HIV Inoculation of Cultures. Placental cultures were maintained as a monolayer for 1 week prior to infection with 1×10^3–1×10^5 tissue culture 50% infective doses of cell-free HIV-1 (IIIB, MN, or BaL strain; Advanced Biotechnologies, Columbia, MD). Alternatively, 1×10^6 phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) infected with HIV-1 and irradiated (8000 rads) were added to cultures. These cells were used for infection; they were stimulated for 48–72 h with PHA, incubated with 10^4 tissue culture 50% infective doses of HIV-1 per 10^7 cells, washed three times in RPMI/10% FCS, and cultured at 10^6 cells per ml in RPMI/10% FCS containing interleukin 2 (2 units/ml) for 1 week prior to irradiation and use as infectious inocula. Cultures incubated with cell-free or cell-associated virus for 16 h at 37°C were washed five times with 5 ml of medium to remove residual inoculating virus or infected cells from exposed monolayers, and fresh medium was added to flasks. Recovery of infected cellular inocula was always >95% of input cells; no nonadherent cells were detected by visual inspection of monolayer cultures. Thereafter, cultures were washed (five times with 5 ml of medium) twice a week, and fresh medium was added to flasks. At each time point, prior to washing and medium replacement, an aliquot of supernatant was frozen for measurement of p24 antigen (Abbott). Cultures were trypsinized and transferred to new flasks once a week for 3–4 weeks. After this, $3–5 \times 10^6$ PHA-stimulated PBMCs (PHA blasts) from uninfected donors were added to rescue latent or undetectable HIV infection. PBMC infection after rescue was measured by weekly sampling of supernatant for p24 without complete washout and replacement of coculture supernatants.

Filter Barrier Inoculation. Placental or HeLa CD4 cells cultured in six-well tissue culture plates for 1 week received 2.5 or 5×10^5 PHA-stimulated, infected, irradiated PBMCs in Cyclopore membrane Transwell cell culture inserts (0.45-μm pore size; Falcon). The next morning membrane inserts containing inocula cells were removed, and placental cells were maintained as described above.

Blocking Studies. Agents tested included anti-Leu-3a (Becton Dickinson) (2.5 μg/ml), anti-LFA-1 (20 μg/ml; a gift from J. Hildreth, Johns Hopkins University, Baltimore), HIV IgG (1:80; a gift from L. Cummins, Abbott), soluble recombinant CD4 (sCD4; 50 μg/ml; Progenics, Tarrytown, NY), and 3′-azido-3′-deoxythymidine (AZT; 0.1 and 1.0 μM). Cell-free virions or HIV-1 infected, irradiated, PHA blasts were preincubated with blocking reagents (except AZT) for 1 h at 4°C. Target monolayers were also preincubated with some reagents (sCD4, anti-LFA-1, anti-Leu-3a, AZT) prior to incubation with cell-free or cell-associated virus inocula in the continued presence of blocking agents during the 16-h coincubation. Monolayers were washed free of blocking agents and inocula and fed with fresh medium containing reagents (anti-Leu-3a, HIV IgG, AZT) at the initial concentrations or (in the case of sCD4) no added reagents. On the fourth day of culture and at all subsequent time points, medium used for washing and feeding contained no blocking agents, with the exception of AZT studies where the drug was maintained in one experimental group through day 15. Cultures were monitored for 3–4 weeks as described above and then cocultured with $3–5 \times 10^6$ PHA blasts in an attempt to rescue latent or undetectable HIV infection over the next 3–4 weeks.

Antibody (Ab) Plus Complement (C)-Mediated Depletion of CD3$^+$ Cells. Monolayers exposed to HIV-1-infected PBMCs and washed as described were incubated 1 day later with 0.1% human IgG for 1 h at 37°C, washed, and incubated with 20 ng of OKT3 (Ortho Diagnostics) in 1 ml medium at 4°C for 30 min. Unbound OKT3 was removed by washing with PBS, and 1 ml of rabbit C (Pel-Freeze Biologicals) diluted 1:4 was added for 1 h at 37°C with shaking of the flask at 15-min intervals. Cultures were then washed three times, refed with culture medium, and incubated at 37°C. In some experiments, parallel placental cultures were treated with OKT3 plus C, 1 day prior to inoculation with infected cells.

Limiting Dilution PCR and Coculture. Monolayers inoculated with cell-associated HIV 7 days previously were trypsinized, counted, and frozen for PCR analysis. At the same time, monolayer cells were plated in 24-well plates at serial dilutions (1×10^5–5×10^1) and cocultured with 1×10^6 PHA blasts. The cultures were fed with RPMI containing 10% FCS and interleukin 2 (2 units/ml) and sampled twice weekly for p24 antigen. Limiting dilution cocultures were similarly performed after OKT3 plus C treatment of monolayers. PCR was performed by a nested primer method using *env*-specific external and internal primer pairs (22).

HIV-1-Specific Ab Detection by Enzyme Immunoassay and Western Blot Assay. Commercial kits for HIV-1-specific Ab detection by enzyme immunoassay (Abbott) and Western blot (Cambridge Biotech) were used according to manufacturers' instructions.

Detection of Active AZT Metabolites. Active phosphorylated AZT anabolites were assayed as described (23).

RESULTS

Phenotype of Cultured Placental Cells. We confirmed many previously reported features of cultured chorionic villus mesenchymal stromal cells (8, 10–12, 24, 25). These adherent cells, which were fibroblastoid in morphology, were 99% homogenous for expression of vimentin. By direct flow cytometry, <1% of the cells expressed surface CD4 or CD14. Immunofluorescence microscopy revealed cells to be <1% CD4$^+$ but 2–5% CD14$^+$ and CD18$^+$ (antigens present to varying degrees on T cells and macrophages). While >90% of the cells expressed esterase and KP1 tissue macrophage protease at varying levels significantly above background or negative control Hela CD4, only 1–3% stained strongly positive, which is consistent with the presence of villous macrophages (Hofbauer cells). All cultures were completely negative for trophoblast-specific human chorionic gonadotropin and human placental lactogen.

Requirements for Transferring Infectious HIV to Placental Cell Cultures and Subsequent Transmission of HIV to PHA Blasts (Rescue). All experiments were repeated at least three times. Initial experiments using 1×10^3–1×10^5 tissue culture 50% infective doses of cell-free HIV-1_{IIIB}, HIV-1_{MN}, or macrophage tropic HIV-1_{BaL} failed to yield productive infection or latent infection that could be rescued with PHA blasts. In contrast, 1×10^6 PHA blasts infected for 2–4 days with any of these HIV strains and then lethally irradiated and placed on placental monolayers for 16 h prior to extensive washing resulted in cultures that were transiently productive of p24 and rescuable 3–4 weeks later by addition of fresh PHA blasts (Fig. 1). The irradiated HIV-infected PHA blasts maintained in parallel did not survive beyond 1 week when cultured at 10^6 cells per ml in the absence of placental stromal cells and stopped releasing new p24 into supernatant by day 10 (data not shown). HeLa CD4$^+$ monolayers supported productive infection with cell-free or irradiated cell-associated HIV-1 (MN or IIIB strain), whereas JAR, HeLa, CCD-18Co, and FHs-738

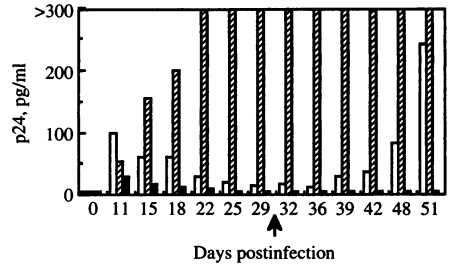

FIG. 1. Supernatant p24 from monolayer cultures exposed to cell-associated HIV for 16 h. HIV-1_{MN}-infected, irradiated (8000 rad) PHA blasts were coincubated for 16 h with placental (open bars), JAR (solid bars), or HeLa CD4 (hatched bars) cells and then removed by extensive washing, trypsinization, and flask transfer. Supernatants were sampled and completely replaced twice weekly. Fresh PHA blasts were added at day 29 (indicated by arrow).

adherent cell lines did not show transiently productive or latent rescuable infection by either mode of inoculation. Furthermore, HIV could not be "rescued" by addition of freshly prepared placental stromal cells.

We measured *de novo* interim release of p24 into completely replaced medium because even after washing out input virus or infected cells to undetectable p24 levels, we consistently obtained transient peaks of "new" supernatant p24 at the next time point assayed. This reequilibration of adherent p24 and/or virus between plastic or cells and fresh medium was removed by the repeated washing, trypsinization, flask transfer, and medium replacement procedure described above, coupled with interim p24 measurements and discarding of the values obtained from the first change of medium (day 3).

Requirement for Cell–Cell Contact. Placental monolayers separated from HIV-1-infected PHA blasts by a 0.45-μm Transwell filter chamber during overnight inoculation failed to support HIV-1 in a form that could be rescued with subsequent exposure to activated PBMCs (Fig. 2). HIV-infected PBMCs remained equally infectious for HeLa CD4 monolayers regardless of the presence of the interposed membrane filter (data not shown).

Inability to Block with Anti-LFA-1 or Anti-CD4 Monoclonal Antibodies (mAbs). Because of the apparent requirement for cell-associated HIV, we attempted to block cell-associated virus with anti-LFA-1 mAb. Fig. 3 illustrates that little or no inhibitory effect was seen at concentrations previously shown to block syncytial fusion between 8E5 cells and PHA blasts (26). To determine the role of CD4 in our system, infected PBMC inocula and placental cells were both preincubated for 1 h with anti-Leu-3a at 2.5 μg/ml, and the same concentration was maintained during the 16-h coincubation and the next 72 h. Little or no blocking of cell-associated HIV-1 was seen (Fig. 3), whereas this concentration of anti-Leu-3a inhibited p24 production by >90% after cell-free HIV infection of susceptible HeLa CD4 cells (data not shown).

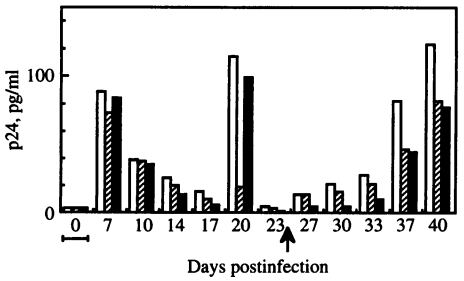

FIG. 3. Minimal effects of anti-Leu-3a or anti-LFA-1 on supernatant p24 from placental stromal cells exposed to cell-associated HIV. Monoclonal anti-Leu-3a (hatched bars) or anti-LFA-1 (solid bars) was added 1 h prior to and during 16 h of coincubation with HIV-infected irradiated PBMC inocula and through day 3 of culture. Control flasks (open bars) received no mAbs. The arrow indicates the addition of fresh PHA blasts. The bar indicates the presence of Ab.

At this point we considered the possibility that apparent transient or latent infection of placental cultures might represent rare, retained inoculum cells sustained by contact with placental cells long enough to transmit HIV to PHA blasts several weeks later. Subsequent experiments were designed to illuminate the nature of this cell-dependent recovery of HIV and distinguish between true placental infection vs. retention and maintenance of infectious inoculum cells.

Ability to Block with Polyclonal Anti-HIV IgG or sCD4. Transfer of cell-associated HIV to placental monolayers was blocked by HIV IgG, which was removed along with overlaid PBMCs. As shown in Fig. 4, inhibition was observed during the 4 weeks of primary cell culture and after addition of fresh PBMCs. To exclude the possibility of residual HIV IgG binding p24, supernatants were tested for Ab by commercial Western blot and enzyme immunoassay and found to be negative by the time PHA blasts were added to cultures. Additionally, immune complex acid dissociation of p24 in culture supernatants did not reveal protein before or after addition of PHA blasts.

Placental cell monolayers preincubated for 1 h with sCD4 at 50 μg/ml and coincubated with infected PHA blasts at this concentration of sCD4 failed to produce p24 after addition of PHA blasts (Fig. 5).

Proportion of Cells in Inoculated Cultures Containing HIV-1 Provirus. Within the inoculated placental monolayer, the frequencies of cells containing HIV-1 proviral DNA and replication-competent provirus were determined by limiting

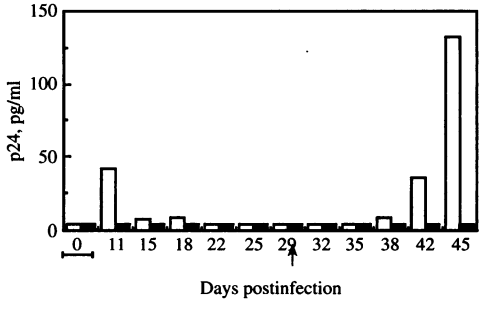

FIG. 2. Requirement for cell–cell contact. Placental monolayers were incubated with HIV-1_{MN}-infected, irradiated PBMCs as described in Fig. 1 (open bars) or were separated from the infected blasts by a 0.45-μm filter membrane (solid bars). The arrow indicates the addition of fresh PHA blasts. The bar indicates the presence of membrane inserts containing inocula for 16–18 h.

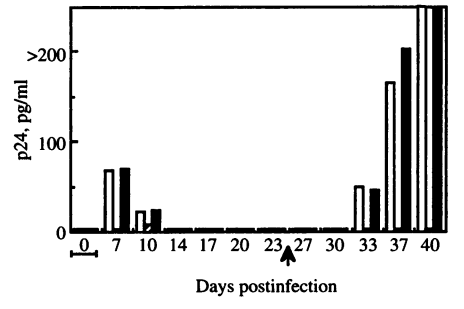

FIG. 4. Polyclonal HIV IgG blocks cell-associated HIV transfer to placental monolayers. Infected inoculum PBMCs and placental cell cultures were pretreated and coincubated in the absence (open bars) or presence of HIV IgG (hatched bars), or normal human IgG (solid bars). No IgG was added after day 3. The arrow indicates the addition of fresh PHA blasts. The bar indicates the presence of Ab.

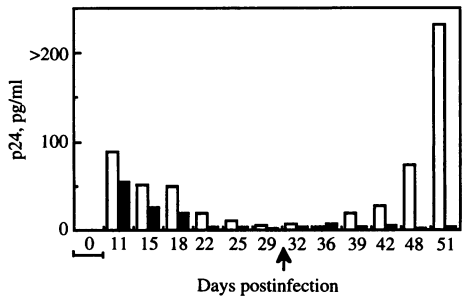

FIG. 5. sCD4 blocks cell-associated HIV transfer to placental monolayers. Infected inoculum PBMCs and placental cell cultures were pretreated and coincubated for 16 h in the absence (open bars) or presence (solid bars) of sCD4 (50 μg/ml); sCD4 was removed along with HIV plus PBMCs by extensive washing and was not added again. The arrow indicates the addition of fresh PHA blasts. The bar indicates the presence of sCD4.

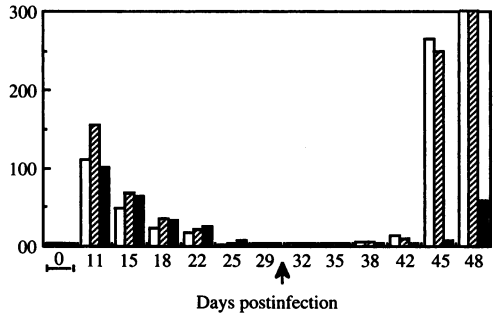

FIG. 6. C-mediated lysis of residual CD3$^+$ cells reduces recovery of HIV. Placental stromal cell monolayers were exposed to HIV-1_{MN}-infected, irradiated PHA blasts for 16 h and washed extensively (open bars). Parallel cultures were treated with OKT3 plus C 1 day before (hatched bars) or 1 day after (solid bars) exposure to infected PBMCs. The arrow indicates the addition of fresh PHA blasts. The bar indicates the presence of OKT3 plus C.

dilution PCR and coculture, respectively (Table 1). Hela CD4 cells repeatedly gave a positive HIV DNA PCR signal at $\geq 5 \times 10^3$ cells, while placental stromal cells were positive only at $\geq 5 \times 10^4$. Coculture analysis was more sensitive than PCR and revealed greater differences in infectious unit (IU) frequencies between infected HeLa CD4 cultures (1 IU per 5×10^2 cells) and placental cells (1 IU per 10^4 cells). JAR cell monolayers exposed to cell-associated HIV were always negative for detectable virus by both PCR and coculture methods.

Anti-CD3 Plus C Treatment. Placental cultures treated once with anti-CD3 and C at 1 or 7 days after cell-associated inoculation showed decreased p24 production after addition of PBMCs 3 weeks later (Fig. 6). HeLa CD4 cultures treated similarly showed no decrease in p24 production compared to untreated controls (data not shown). After C-mediated lysis of CD3$^+$ cells, serial 5-fold limiting dilution cocultures of infected placental cells typically showed a 10-fold or greater reduction in the frequency of recovered IUs (Table 2). When placental cells were similarly treated 24 h prior to inoculation, to deplete preexisting fetal or maternal T lymphocytes from culture, there was no reduction in recovered HIV (Fig. 6).

AZT Treatment. HPLC analysis of phosphorylated anabolites showed similar utilization of AZT by placental cells, HeLa CD4, and PHA blasts incubated *in vitro* with tritiated drug. However, AZT present before, during, and for several weeks after coincubation of placental cells with infected cells, at concentrations inhibitory for HeLa CD4 infection, failed to significantly decrease recovery of HIV at time of rescue (Table 3).

DISCUSSION

Mechanisms of HIV transmission from infected mother to unborn child cannot be studied *in vivo*, and appropriate animal models are lacking. Many recent *in vitro* models have focused on trophoblast-derived cell lines, which may not reflect HIV susceptibility of primary placental cells, particularly those of the mesenchymal stroma. Studies using placental explants may suffer from problems related to difficulties in controlling for input p24 and/or viral DNA (misinterpreted as transient *de novo* HIV production).

The fetal placental stromal cells are in direct contact with the fetal blood and constitute the final barrier that must be breached or infected for HIV transmission to occur. Maternal and fetal macrophage-like cells may migrate between stromal and trophoblast layers, facilitating infection. We therefore developed a primary cell culture model for the interaction of fetal placental stromal cells with infected PBMCs. These mesenchymal cells constitute up to 50% of the intact chorionic villi, have features consistent with both macrophage function (phagocytosis and lysosomal enzymes) and connective tissue (vimentin and fibronectin), and were not readily infectable with various strains of cell-free HIV, including a macrophage tropic strain. Cell-associated HIV was retained by these cultures in a manner requiring intimate cell–cell contact but not CD4 binding or observable syncytia formation.

A gp120 binding high-mannose receptor is a candidate placental non-CD4 receptor (27), and recent studies document cell-associated transmission of HIV without requirement for specific viral receptors (18, 28, 29). Electron micrographs revealed polarized release of HIV particles from infecting cell processes invaginating the surface of cells being infected.

In spite of these precedents for CD4-independent, cell-associated HIV infection, we interpret our data as demonstrating a mechanism that differs from true infection. The fact that a very limited number of cells contained provirus or could serve as infectious foci after exposure to cell-associated HIV and the inability to rescue productive infection with freshly added placental cells suggest that there was no spreading

Table 1. Limiting dilution HIV PCR and coculture

	Placental stromal cells		HeLa CD4		JAR	
No. of cells	PCR	p24	PCR	p24	PCR	p24
1×10^5	+	+++	+	+++	−	−
5×10^4	+	+++	+	+++	−	−
1×10^4	−	++	+	+++	−	−
5×10^3	−	−	+	+++	−	−
1×10^3	−	−	−	+	−	−
5×10^2	−	−	−	+	−	−
1×10^2	−	−	−	−	−	−

The indicated numbers of cells were cocultured with 10^6 uninfected PHA blasts and sampled for supernatant p24 at 3- to 4-day intervals: +++, p24$^+$ by day 3; ++, p24$^+$ by day 7; +, p24$^+$ by day 14; −, p24$^-$ through day 30.

Table 2. Limiting dilution coculture of placental stromal cells: Effect of C-mediated cytolysis of CD3$^+$ cells

No. of cells	Untreated	OKT3 + C
1×10^5	+++	+/−
5×10^4	+++	−
1×10^4	+/−	−
$0.1–5 \times 10^3$	−	−

Purified placental stromal cells exposed for 16 h to 10^6 HIV-infected PBMCs and washed free of nonadherent inoculum cells were treated with OKT3 (20 ng/ml) + C, washed, and maintained for 3–4 weeks, at which time limiting dilution cocultures were established and scored as in Table 1: +++, p24$^+$ by day 3; +/−, p24$^+$ by day 21; −, p24$^-$ through day 30.

Table 3. p24 production 10 days after PHA blast rescue of inoculated cultures

Cells	AZT			p24	
	Conc., μM	Pre	Post	Conc., pg/ml	%
Placental	0	−	−	1,340	100
	0.1	+	−	1,940	144
	1.0	+	−	1,280	95
	1.0	+	+	622	46
HeLa CD4	0	−	−	13,260	100
	0.1	+	−	614	4.6
	1.0	+	−	165	1.2
	1.0	+	+	66	0.5

Placental and HeLa CD4 cells were incubated with (0.1 or 1.0 μM) or without AZT before (pre) or during and after (post) coincubation with HIV-infected cells. The p24 concentration was determined and was expressed as a percentage of the p24 concentration in the supernatant of cells that received no AZT.

infection within such cultures. Inability to induce even latent infection with 10^5 tissue culture 50% infective doses of cell-free virions or 5×10^5 infected PBMCs separated by a virus-permeable filter and the absence of blocking by anti-CD4 do not support the idea that rare $CD4^+$ placental cells are the source of infectious foci.

Rather, reduction in infectious foci and rescue of HIV from these cultures by treatment with anti-CD3 plus C after, but not before, exposure to infected cells points to rare residual HIV-infected inoculum T cells as the source of recoverable virus in these cultures. Increased type IV collagenase activity and basement membrane invasiveness (30) of HIV-infected lymphocytes may enhance the ability of such cells to become embedded in and sustained by the placental stromal cell monolayer.

Appropriately metabolized AZT, present at inhibitory concentrations throughout coincubation of placental cells with infected cells, failed to significantly decrease recovery of HIV at the time of rescue. This finding implies that new infection of placental cells is not occurring and is consistent with retained infected cells acting as the source of recovered virus.

Because we originally thought that placental cells would be susceptible to true infection, we used lethally irradiated lymphocytes so that their contribution to observed HIV production over time would be minimal. Retention of these lymphocytes with the ability to transmit HIV after 4 weeks suggests a "nurse cell" role for placental cells in delaying radiation-induced apoptosis (31). Synovial fibroblasts have previously been shown to support T cells in a nondividing state after removal of interleukin 2, which would otherwise result in T-cell apoptosis (32). The transient release of p24 into culture supernatants was probably from viable and dying inoculum cells. Since cytotoxic T lymphocytes are thought to deliver lytic signals by triggering apoptosis in targets (33–35), our findings may have implications for the ability of HIV-specific cytotoxic T lymphocytes to eliminate intraplacental infected cells. Additionally, HIV-induced apoptosis of infected cells (36) may be inhibited when these cells are in intimate contact with placental tissue, thereby prolonging their period of potential HIV transmission to the fetal circulation.

The ability to retain infected cells in placental cell cultures and recover HIV with fresh PBMCs after several weeks satisfies key criteria for transplacental transmission without postulating true infection of placental mesenchymal stromal cells. The blocking effect of HIV IgG is consistent with a requirement for intimate contact between uninfected placental cells and HIV-infected lymphocytes expressing viral antigens on their surface. This suggests a role for maternal Abs in blocking HIV transmission *in utero* and may support the concept of passive immunization during pregnancy to increase protective Ab levels.

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