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Human Immunodeficiency Virus Type 1 Infection of Human Placenta: Potential Route for Fetal Infection

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To determine the potential role of the placenta in transmission of human immunodeficiency virus (HIV) from mother to fetus, the ability of human placental tissue to support HIV type 1 (HIV-1) infection was examined. HIV-1-seronegative first-trimester placentas were maintained in culture and infected with HIV-1. Virus production, measured by HIV-1 antigen release into the supernatant, and HIV-1 DNA, identified by polymerase chain reaction, were detected for at least 12 days postinfection. Western immunoblot analysis showed Gag proteins, precursor p55, and cleavage products p24 and p17 in HIV-1-infected tissues. Double labeling of placental villi with antibodies to CD4 and placental trophoblast-specific alkaline phosphatase indicated that trophoblasts express CD4 antigen. Additionally, immunostaining of HIV-1-infected tissues with anti-p24 antibodies demonstrated HIV-1 protein expression in placental trophoblasts. Evaluation of human chorionic gonadotropin and progesterone production by the placental cultures indicated that there was a 90% decrease in human chorionic gonadotropin and a 70% decrease in progesterone production in HIV-1-infected cultures in comparison with controls. These data demonstrate that trophoblastic cells of human placenta tissue express CD4 and are susceptible to HIV-1 infection; also, placental endocrine function is decreased by HIV-1 infection. Thus, the placenta may serve as a reservoir of HIV-1 infection during pregnancy contributing to infection of the fetus, and decreased placental hormone production may result in impaired fetal development.

The number of women infected with human immunodeficiency virus (HIV), the causative agent of AIDS, is increasing, and the majority of these women are of child-bearing age (3, 6, 17). Approximately 30% of the infants born to infected mothers will have evidence of HIV infection or AIDS by the age of 18 months, and 20% of infected children will have died by that time (2). Therapeutic and prophylactic interventions designed to interrupt transmission of HIV from an infected woman to her newborn are essential to prevent children from infection. Critical for the development of the treatment(s) necessary to prevent mother-infant transmission is an understanding of the pathogenesis of HIV infection in pregnant women and newborn infants. However, there is conflicting information regarding the effect of HIV infection on pregnancy (5, 14, 22) as well as the routes of perinatal transmission (8–10, 15, 18, 20, 21, 24). Although transplacental transmission of HIV is generally accepted, little research has focused on the pathogenesis and risk factors associated with early transmission, the role of the placenta in perinatal transmission, or placental damage and physiological dysfunction associated with HIV infection. Identification of the role of the placenta in modifying HIV transmission may lead to the development of important strategies designed to prevent placental infection and subsequent transplacental transmission to the fetus. In this report, human placental cells are demonstrated to express CD4, to be permissive for HIV type 1 (HIV-1) infection, and when infected with HIV-1, to produce markedly decreased amounts of placental hormones.

MATERIALS AND METHODS

Placental culture. First-trimester placentas were obtained from HIV-1-seronegative women undergoing elective abortion by vacuum aspiration. The tissues were immediately transferred into 100 ml of cold sterile phosphate-buffered-saline (PBS) containing streptomycin (1,000 μg/ml), amphotercin (25 μg/ml), and nystatin (1,000 μg/ml), shaken vigorously, and transferred to a second bottle containing the same solution. This transfer and rinsing process was repeated four times. Then explant cultures were established in Eagle minimal essential medium (E-MEM) containing 10-fold dilutions of the above antibiotics and incubated at 37°C on a rocker for 12 days as previously described (1), with the following modifications. For each experiment, 24 1-g each portions of placental tissue were cultured and maintained separately in four six-well tissue culture plates (Costar, Cambridge, Mass.). The explants of two plates were used as uninfected control samples, and explants of the other two plates were infected with HIV-1. To prevent placental explants from losing their integrity in culture medium, the morphology of villi in organ culture was evaluated by sequential phase-contrast and light microscopy over the 12-day duration of culture.

Hormone production in infected explant cultures. As an indicator of the viability and function of placental explants, at 24-h intervals following initial culture, an aliquot of culture medium was frozen at −70°C and later assayed for the secretion of human chorionic gonadotropin (hCG) and progesterone, using commercially available radioimmunoassay kits (ICN, Horsham, Pa., and Bioquant Inc., Ann Arbor, Mich., respectively). Aliquots of culture media from human foreskin fibroblast cultures as well as E-MEM supplemented with 10% fetal calf serum were used as controls. To eliminate the possibility of the presence of residual hormones in culture medium during the 12-day incubation period, the placental explants were washed with PBS and reincubated in fresh medium every 24 h.

For studies designed to evaluate hormone production in HIV-1-infected placental explants, media from infected and

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uninfected cultures were examined for hCG and progesterone as described above.

Immunofluorescence double labeling. To determine whether placental cells express CD4 antigen, frozen sections of first-trimester human placentas were incubated with mouse anti-CD4 monoclonal antibodies (anti-Leu 3a and anti-Leu 3b; Becton Dickinson, Mountain View, Calif.) and rabbit antiplacental alkaline phosphatase (obtained from J. Millan, La Jolla Cancer Institute, La Jolla, Calif.) shown to be specific for human placental trophoblasts (13). Species-specific secondary antibodies were labeled with biotin and linked with fluorescein isothiocyanate-avidin for identification of CD4 antigen and with rhodamine isothiocyanate-avidin for detection of the trophoblastic marker. Negative controls consisted of frozen sections of placentas treated with a control mouse monoclonal antibody (anti-keyhole limpet hemocyanin immunoglobulin G1; Becton Dickinson) and control rabbit antiserum (anti-normal rat serum; Kallestad, Chaska, Minn.).

Assay for virus production. To determine whether placental cells are permissive for HIV-1 infection, each explant culture was infected with 4 ml of filtered LAV-BRU strain of HIV-1 (4×10^4 50% tissue culture infectious doses per culture). Following virus adsorption, each culture was washed five times with PBS. Additionally, at 24-h intervals, the placental explants were washed at least three times to remove free virus and resuspended in fresh medium. Twenty-four hours after each wash, supernatants were collected and monitored daily, using the Abbott HIV-1 antigen enzyme immunoassay. To evaluate the infectivity of released virus, 4 ml each of filtered HIV-1-infected and mock-infected culture media was added to a flask containing 5×10^6 phytohemagglutinin-stimulated peripheral blood mononuclear cells. The supernatants were tested for the presence of p24 antigen for up to 28 days.

Amplification of HIV-1 DNA sequences in infected placental cells. To determine whether HIV-1 DNA replication occurred in placental cells, total cellular DNA was extracted from HIV-1-infected placental explants at designated times postinfection. One microgram of each denatured DNA sample was used for in vitro amplification by the polymerase chain reaction (PCR), using primers SK 38 and SK 39 from the *gag* region of HIV-1 (16). Samples of uninfected placental tissue, HIV-1-infected CEM cells, uninfected CEM cells, and ACH2 cells (4) were used as controls. Aliquots of 10% of the PCR reaction products were denatured and subjected to liquid hybridization with specific ^{32}P-end-labeled SK 19 probe (16) and analyzed in 10% polyacrylamide gels as described elsewhere (7).

Western immunoblot analysis. To examine the expression of HIV-1 proteins in infected placental explants, tissues were harvested and washed with PBS at 48-h intervals from 2 to 12 days postinfection. Concentrations of protein samples extracted from HIV-1-infected and control tissues were measured with colloidal gold reagent (Integrated Separation System, Hyde Park, Mass.) according to the procedures recommended by the manufacturers. A 2-μg sample of each protein was analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose (26). Proteins from uninfected placental tissue and from infected CEM cells were used as controls. Blots were reacted with specific antibodies as described elsewhere (23), with the following modifications. Membranes were incubated in blocking buffer (950 g of dry milk in PBS plus 0.5 ml of anti-Fome A at 4°C). After 24 h, the membranes were reacted with a 1:100 dilution of mouse monoclonal antibody to the Gag proteins (anti-p24; Dupont, Wilmington, Del.) in blocking buffer for 24 h at 4°C, rinsed twice with Tris-buffered saline (TBS), and incubated in a 1:1,000 dilution of rabbit anti-mouse immunoglobulin G (Jackson Immuno Research, West Grove, Pa.) in blocking buffer for 1 h at room temperature. Then blots were washed twice with TBS and three times with blocking buffer for 30 min each time and incubated with 25 ml of 1 μCi of ^{125}I-labeled protein A per ml in blocking buffer for 1 h at room temperature. Following incubation, the blots were rinsed twice with TBS, washed three times with blocking buffer for 30 min each time, and then rinsed once with TBS and once with water. Subsequently, blots were dried at room temperature and autoradiographed at −70°C with intensifying screens.

Immunostaining of HIV-1-infected placental explants. To demonstrate the expression of HIV-1 protein in infected cells, rabbit polyclonal antibody to HIV-1 p24 protein (Microgenesys, Inc., West Haven, Conn.) was used for immunohistochemistry studies. Paraffin sections were incubated with 10% normal goat serum for 1 h at room temperature and then with polyclonal antibody at a dilution of 1:1,000 for 24 h at 4°C. Sections were washed with Tris buffer (pH 7.4) and then incubated for 30 min at room temperature with biotinylated anti-rabbit immunoglobulin G diluted to 1:100 (Dako, Carpinteria, Calif.). After further washing, the sections were incubated with avidin-biotin-horseradish peroxidase (Dako). The slides were developed with aminoethylcarbonate substrate. Negative controls consisted of infected placental tissue sections treated with rabbit antiserum to normal rat serum and uninfected chorionic villi, treated with rabbit anti-p24 antibody.

RESULTS

Morphological and biosynthetic evaluations of placental explants. In organ culture, the placental tissues maintained a normal cellular morphology and organization that was similar to those of samples examined as fresh tissue before incubation. An additional indicator of the viability and function of the placental explants was obtained by measuring the ability of the trophoblastic cells to synthesize and secrete hCG and progesterone hormones. At designated time intervals during the 12-day culture period, aliquots of media from placental explants, of control samples of foreskin fibroblast culture, and of E-MEM were assayed for hCG and progesterone concentrations. When undiluted media from placental cultures were tested by quantitative radioimmunoassay procedures, they contained hCG at a concentration of greater than 100 mIU/ml and progesterone at a concentration of 8 to 10 μg/ml. Control samples contained hCG at <10 mIU/ml and progesterone at <1 μg/ml.

Expression of CD4 antigen in trophoblastic cells. To assess the potential for HIV-1 entry into placental cells, immunohistochemical studies were conducted to determine whether CD4 antigen was present on the surface of cells in explant culture. Frozen sections of first-trimester placenta were stained with mouse anti-CD4 and rabbit antiplacenta alkaline phosphatase. The predominant cells in placental villa which stained positively with both antibodies were trophoblastic cells (Fig. 1). Placental villi treated with control mouse monoclonal and control rabbit sera showed no staining (data not shown).

Virus production in placental explants. To detect the release of HIV-1 antigens by infected placental cells, aliquots of growth medium from infected explants were examined by the Abbott HIV-1 antigen enzyme immunoassay. Although

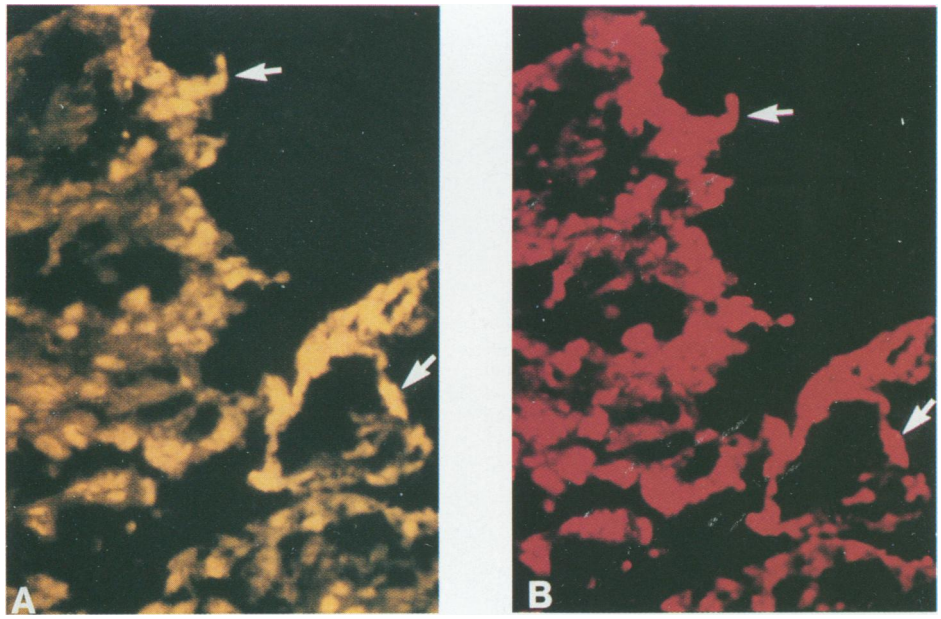

FIG. 1. Expression of CD4 antigen by trophoblastic cells (arrows) as detected by immunofluorescence double labeling. Frozen sections of first-trimester placenta were stained with monoclonal antibodies directed against CD4 antigen (A) and rabbit antiplacenta alkaline phosphatase specific for placental trophoblasts (B). Trophoblastic cells stained positively with both antibodies.

there was a progressive decline in the quantity of p24 antigen released in culture medium over time, placental tissue supported HIV-1 infection, with production of more than 100 pg of HIV-1 antigen per ml for up to 12 days (Fig. 2). Additionally, when cocultivated with phytohemagglutinin-stimulated peripheral blood mononuclear cells, supernatants obtained from HIV-infected explant cultures produced HIV-1.

HIV-1 DNA in infected placental cells. To demonstrate that the low and decreasing amount of p24 antigen released into the supernatant was not due to the presence of residual virus inoculum and that the HIV-1 RNA genome was actively transcribed into DNA in infected samples, we analyzed DNA extracted from HIV-1-infected and uninfected samples by PCR. Figure 3 demonstrates the presence of the amplified 115-base fragment of HIV-1 DNA in infected samples from 2 to 12 days postinfection.

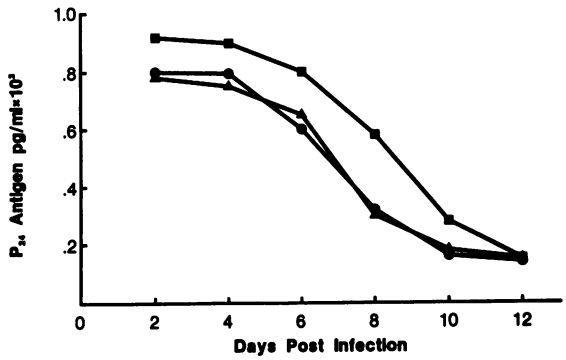

FIG. 2. Production of HIV-1 antigens in the supernatant of placental explants infected with HIV-1 as described in Materials and Methods. At 24 h after each wash, supernatants were collected, and the samples were monitored every other day by using the Abbott HIV-1 antigen enzyme immunoassay. Placental tissue supported HIV-1 infection, with production of >100 pg of HIV-1 antigen per ml for up to 12 days. Shown are results of three representative experiments.

Expression of HIV-1 Gag proteins in infected explants. Expression of HIV-1 proteins in infected placental cells was determined by Western blot analysis of proteins extracted from HIV-1-infected placental tissue. Protein extracts were prepared at 48-h intervals up to 12 days postinfection. Extracts of uninfected placental explants and HIV-1-infected CEM cells were used as controls. The Gag precursor (p55) and some evidence of Gag cleavage products (p24 and p17) were present in HIV-1-infected placental tissues from 2 to 12 days postinfection (Fig. 4).

To further identify which cells within the placenta were expressing HIV-1 Gag proteins, paraffin sections of infected explants were evaluated by immunocytochemistry. By using the rabbit antibody to HIV-1 Gag proteins, cells in the chorionic villous tissue were shown to express HIV-1 proteins. Positive cells were seen predominantly in the villous trophoblastic layers (Fig. 5) as early as 48 h postinfection

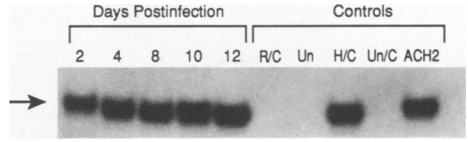

FIG. 3. Amplification of DNA extracted from HIV-1-infected placental explants at 2, 4, 8, 10, and 12 days postinfection. Samples of reagent control (R/C), uninfected placental tissue (Un), HIV-1-infected CEM cells (H/C), uninfected CEM cells (Un/C), and ACH2 cells were used as controls. A 1-μg amount of each denatured DNA sample was used for PCR, using primers SK 38 and SK 39 from the *gag* region of HIV-1. Aliquots of 10% of the PCR reaction products were denatured and subjected to liquid hybridization with a specific ^{32}P-end-labeled (SK 19) probe and analyzed in 10% polyacrylamide gels. The arrow marks the resulting amplified fragment (115 bases) that indicates the presence of the specific HIV-1 amplified product.

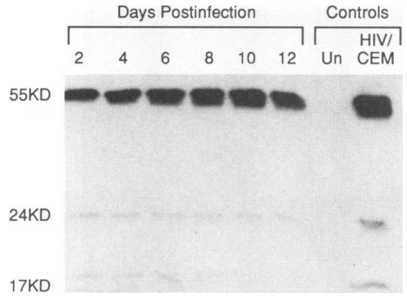

FIG. 4. Western blot analysis. Immunoblot analysis of proteins extracted from HIV-1-infected placental tissue at 2, 4, 6, 8, 10, and 12 days postinfection was performed by using a mouse monoclonal antibody to Gag proteins. Uninfected placental samples (Un) and HIV-1-infected CEM cells (HIV-1/CEM) were used as controls. Gag precursor protein (p55) and some evidence of cleaved p24 and p17 were detected in HIV-1-infected placental tissue. KD, Kilodaltons.

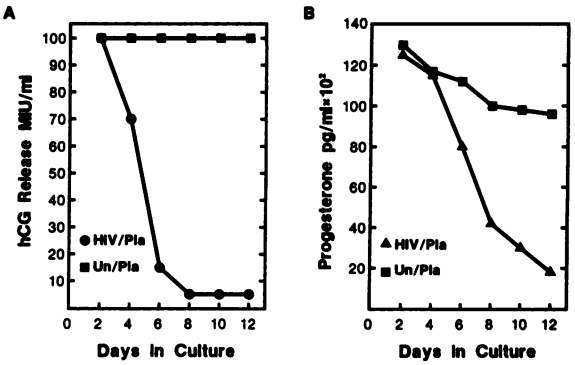

FIG. 6. Hormone secretion by HIV-1-infected placental explants. At designated time intervals postinfection, aliquots of media from HIV-1-infected explants (HIV-1/Pla) were assayed for hCG (A) and progesterone (B) secretion. A decrease of 95% in hCG concentrations and 70% in progesterone concentrations was observed in comparison with uninfected controls (Un/Pla) of equal weight.

(data not shown) and persisted throughout the 12-day experimental period. HIV-1 antigens were also detected in Hofbauer cells (villous macrophages). Mock-infected control tissues treated with positive primary antibody and infected samples treated with nonrelevant antibody did not exhibit positive staining.

Pattern of hormone production in HIV-1-infected placental explants. The effect of HIV-1 infection on placental function was determined by analyzing media from HIV-1-infected and uninfected placental explants for hCG and progesterone secretion (Fig. 6). By 4 days postinfection, there was a greater than 30% decrease in hCG production, which continued to decline and reached less than 95% of the control value by 8 days postinfection. Progesterone production in infected placentas declined more slowly than hCG production. However, there was a consistent reduction in progesterone produced by HIV-1-infected placental explants, which was 30% of the control value by 12 days postinfection.

DISCUSSION

Women and children are the most rapidly expanding groups of individuals infected with HIV-1. Although the proportion of infants who acquire HIV-1 infection in utero versus intrapartum is unknown, increasing evidence supports placental transmission as an important route of fetal infection. The relative risk of HIV-1 infection during different stages of gestation is unknown. However, because the placental trophoblast is the only fetal cell type that is exposed to the maternal uterine decidua and blood, it is likely that if infection occurs at any time during gestation, these cells would serve as an important site of HIV-1 infection.

In this report, we describe the use of placental explant cultures as a model of HIV-1 infection of human placental tissue. Using this system, first-trimester trophoblastic cells

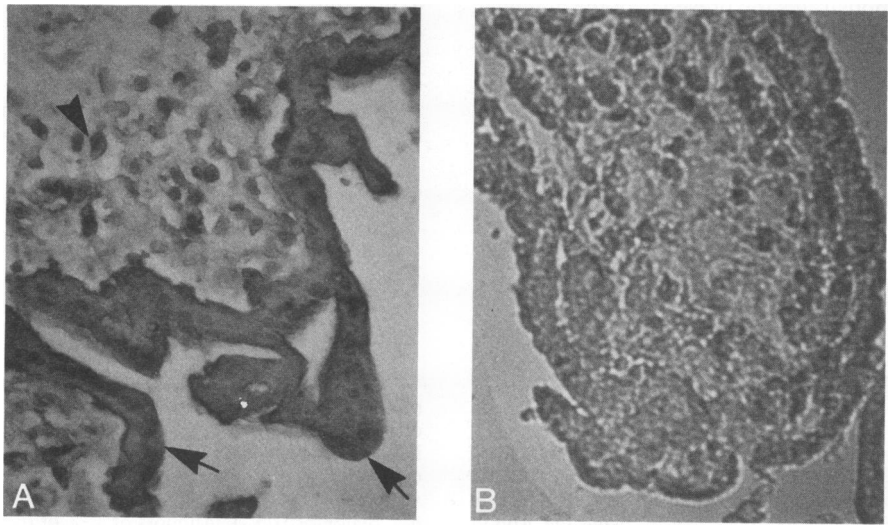

FIG. 5. Tissue staining. Paraffin sections of HIV-1-infected (A) and uninfected (B) tissues were incubated with primary rabbit antibody to HIV-1 p24 and treated with streptavidin-biotin conjugates. Trophoblastic cells of the chorionic villi showed positive staining (arrows). HIV-1 antigens were also detected in Hofbauer cells (villous macrophages; arrowhead). Mock-infected control tissues treated with positive primary antibody did not exhibit positive staining.

are demonstrated to express CD4 antigen and thus provide potential targets for HIV-1 infection. Infection of these placental cells is shown by the release of HIV-1 antigens into culture supernatant and the detection of HIV-1 DNA and the presence of HIV-1 Gag proteins in infected tissue. These studies confirm and expand the findings recently reported by Maury et al. (12), who despite their inability to demonstrate the production of virus as detected by reverse transcriptase activity could demonstrate the expression of HIV-1 antigens in infected placental tissue. The decreased production of hCG and progesterone in infected explant cultures in our studies provides further evidence for direct infection of the trophoblastic cells.

The demonstration that first-trimester human placental tissue can be infected by HIV-1 supports the pathologic and virologic findings that HIV-1 can be transmitted early in gestation (8, 9, 24). However, the extent of placental infection and the relative ability of early versus late placental tissue to support HIV-1 infection are unknown. Additionally, no information is available as to whether certain strains (lymphotrophic versus monocytotropic, lytic versus nonlytic, syncytium forming versus non-syncytium forming, etc.) are more likely to infect placental tissue with subsequent transmission to the fetus. These studies could provide important insights into the pathogenesis of HIV-1 infection and help in the development of treatment strategies designed to interrupt mother-infant transmission.

The finding that levels of hCG and progesterone are markedly decreased in HIV-1-infected placental cells has important implications for the possible effects of HIV-1 on the developing fetus. In this regard, several reports have described prematurity and low birth weight as well as developmental abnormalities to be associated with maternal HIV-1 infection (11, 21, 25), while others have found no such association (2, 19). Because the health status of the infected mother, substance abuse, and other maternal risk factors can contribute to low birth weight and developmentally impaired newborns, well-controlled studies will be necessary to identify the true association of HIV-1 with fetal development.

The experiments summarized in this report were reproducible with use of several placental explant cultures obtained from different women. It is unlikely, therefore, that specific differences exist at the placental level which determine the susceptibility of a specific placenta to HIV-1 infection. However, it is possible that maternal virus load and immunologic status and placental contact with HIV-1-infected macrophages of maternal decidua are determinants of placental infection and subsequent viral transmission to the fetus. If in utero infection is dependent on placental infection, recognition of an affected placenta could provide a mechanism for identification of at-risk infants subsequently identified as HIV-1 infected. Further studies which identify the factors relating to HIV-1 infection of the placenta and subsequent transplacental transmission are necessary to determine these factors.

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