Short Communication

Up-Regulation of CCR5 Expression in the Placenta Is Associated with Human Immunodeficiency Virus-1 Vertical Transmission

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The role of placenta in vertical transmission is not yet fully understood. A protective role of the placenta during gestation is suggested by the finding that caesarian sections reduce the risk of transmission of human immunodeficiency virus (HIV)-1 from mother to child three- to fourfold. Here we investigated whether the immunological milieu of the placenta might be important in HIV-1 transmission. *In situ* imaging of immunohistochemically stained placenta sections and reverse transcriptase-polymerase chain reaction demonstrated a fourfold increase in CCR5:CXCR4 expression ratio in placentae from transmitting women compared to placentae from nontransmitting women. This chemokine receptor repertoire was consistent with an up-regulation of interleukin-4 and interleukin-10 expression in placentae from nontransmitting placentae compared to transmitting placentae. *In situ* imaging demonstrated that CCR5 and CXCR4 were expressed on placental macrophages and lymphocytes but not in trophoblasts. Simultaneous immunofluorescence/ultrasensitive *in situ* hybridization for HIV-1 *gag-pol* mRNA revealed that HIV-1 infects primarily CXCR4-expressing cells in placentae from nontransmitting women whereas predominantly CCR5-expressing cells were infected in placentae from transmitting women. These data are consistent with transmission of a homogeneous population of nonsyncytium-inducing HIV-1 isolates that use CCR5 as co-receptor. (Am J Pathol 2000, 157:1811-1818)

Vertical transmission of human immunodeficiency virus (HIV)-1 occurs in ~14 to 39% of pregnancies unless antiretroviral treatment is provided.^{1,2} The neonatal infection rate is reduced to ~8 to 9% if zidovudine (AZT) treatment is implemented throughout the perinatal period.³ Elective caesarian sections also reduce the risk of transmission of HIV-1 from mother to child three- to fourfold independently of zidovudine treatment, supporting a role of the placenta in preventing HIV-1 transmission during gestation.⁴

The placenta is composed of fetal capillaries, placental macrophages (Hofbauer cells), and cytotrophoblasts surrounded by syncytiotrophoblasts to form a functional subunit, the placenta villous. The barrier between maternal and fetal circulation (and ultimately HIV-1 transmission) is several cell layers thick and consists of syncytiotrophoblasts, Hofbauer cells, and intervening stroma. It is controversial whether syncytiotrophoblasts are susceptible to HIV-1 infection although syncytiotrophoblasts were shown to be infected *in vitro* by co-culture with virus-infected maternal lymphocytes; a process enhanced by antibody.⁵⁻⁸ In another study, trophoblasts grown in primary culture were, however, not infectable by free virus but supported replication of virus introduced by transfection.⁹ Given that syncytiotrophoblasts may not be infectable by free virus, other mechanisms such as structural disruptions in the syncytiotrophoblasts, endocytosis of viral particles, or active transport of HIV-1 immune complexes via Fc or complement receptors have been proposed for transplacental passage of HIV-1.¹⁰⁻¹² Functional gene transfer of HIV-1 DNA by uptake of apoptotic bodies, independently of free virus and HIV-spe-

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cific receptors, was recently demonstrated.¹³ This mechanism might be another alternative to explain transplacental passage/infection of syncytiotrophoblasts. After penetrating the trophoblast layer, HIV-1 encounters cells expressing HIV-1 co-receptors such as lymphocytes and macrophages. Placental macrophage-like cells (Hofbauer cells) support HIV-1 replication of both CCR5-using (R5) and CXCR4-using (X4) isolates as well as primary isolates.^{14,15}

Numerous studies comparing maternal HIV-1 isolates to those transmitted to their children reveal transmission of a selected, homogeneous population of HIV-1 isolates^{16–18} although not all agree.¹⁹ Although most studies agree that some form of selection occurs during vertical transmission, few studies have identified the placenta as being involved in this selection process. No studies to date have identified the mechanism underlying the selection process in the placenta. Here, we investigate one possible mechanism for HIV-1 selection in the placenta.

Previously, we have shown *in vitro* and *in vivo* that chemokine receptor expression on immune cells is regulated by type 1 and type 2 cytokines.^{20–22} Type 1 cytokines such as interferon- γ or interleukin (IL)-2 up-regulate both CCR5 and CXCR4 mRNA and protein expression whereas Type 2 cytokines such as IL-4 and IL-10 up-regulate CXCR4 mRNA and protein expression without affecting or decreasing CCR5 mRNA or protein expression.²¹ We hypothesize that this mechanism effectively functions in the placenta.

Here we describe profound differences in chemokine receptor expression between placentae from HIV-1 transmitting and nontransmitting women. These different expression patterns can be attributed to distinctly different cytokine milieus in transmitting placentae compared to nontransmitting placentae. We also demonstrate that differential chemokine receptor expression selects for productive infection of cell type expressing the predominant receptor. This selection based on chemokine receptor expression is consistent with the predominance of R5, nonsyncytium-inducing isolates in perinatally infected children.

Materials and Methods

Study Participants

Placenta samples were obtained from 23 HIV-1-infected women including 16 from nontransmitting women (TNT) and seven placentae from transmitting women (TT). The tissues were obtained from women at the time of normal vaginal delivery after consent. In addition, term placentae from HIV-1-seronegative women devoid of inflammation or other pathology were used as normal controls. TT women were matched with TNT women for maternal CD4 count, placenta gestational age, and age. None of these women had any antiretroviral therapy during pregnancy. Only women with the w/w CCR5 genotype were selected for this study. The Ethical Review Committee approved the study protocol.

Tissue and Cell Preparation

Tissue samples were homogenized for RNA extraction, snap-frozen in OCT embedding compound, or fixed in Streck tissue Fixative (Streck Laboratories, Omaha, NE). RNA was extracted from biopsy specimens by homogenizing fresh biopsies of 5 mm³ in 500 μ l of TriReagent using diethyl pyrocarbonate-treated, autoclaved, disposable homogenizers. After homogenization RNA was purified as per the manufacturer's protocol. RNA pellets were resuspended in 1 $\times$ transcription buffer (Promega, Madison, WI) with 2 units RQ1 RNase-free DNase. DNA was digested for 30 minutes at 37°C to remove contaminating DNA. The mixture was extracted once with phenol:chloroform:isoamyl:alcohol, and once with chloroform:isoamyl:alcohol. The aqueous layer was removed and the RNA was precipitated in 3 volumes of ethanol and 1/40 volume of 3 mol/L of sodium acetate overnight at –20°C.

Immunohistochemistry/in Situ Image Analysis

Tissue sections were cut to 8 μ m, adhered to silanized slides, and deparaffinized through xylenes and graded alcohols. After peroxidase quenching and blocking with mouse serum in phosphate-buffered saline (PBS), pH 7.4, with 5% nonfat dry skim milk, immunohistochemistry was performed using the Vectastain ABC-HP kit (Vector Laboratories, Burlingame, CA) as per the manufacturer's recommendations. Diaminobenzidine was used as substrate with hematoxylin counterstain. Frozen tissue sections for quantitative image analysis were allowed to air-dry for 15 minutes and were followed by postfixation in 2% formaldehyde for 20 minutes. Sections were washed in PBS and an optimized dilution of primary antibody was applied. Commercially available antibodies to IL-2, IL-4, IL-10 (PharMingen, San Diego, CA) were used at concentrations optimized on control tissues. Quantitative assessments of the staining for chemokine receptors CCR5 (45549.11/45531.111 mouse IgG2b) and CXCR4 (44717.111, mouse IgG2b) were obtained from R&D Systems (Minneapolis, MN). The sections were stained without the primary mAb to control for nonspecific background. Irrelevant isotype-specific antibodies; rabbit anti-mouse IgG1, IgG2b, and rabbit anti-rat were used to control for nonspecific staining reactions. The staining procedure was performed as previously described.²³ Cytokine and chemokine expression was quantified using *in situ* image analysis as previously described.²³

Chemokine Receptor and Cytokine mRNA Quantification

Quantitative real-time reverse transcription polymerase chain reaction was performed by adding 45 μ l of the reaction mix, which includes 1 $\times$ RT Taqman EZ buffer (PE Applied Biosystems, Foster City, CA), 4.0 mmol/L Mn(OAc)₂, 300 μ mol/L dATP, 300 μ mol/L dCTP, 300 μ mol/L dGTP, 300 μ mol/L dTTP, 200 nmol/L upstream primer, 200 nmol/L downstream primer, 200 nmol/L internally-conserved fluorogenic probes, and 10 units rTth

Table 1. Quantification of mRNA in TT, TNT, and Normal Control Placentae

Placenta type	n	Chemokine receptor mRNA (copies/20,000 GAPDH copies)			Cytokine mRNA (copies/20,000 GAPDH copies)		
		CCR5*	CXCR4 [†]	CCR5:CXCR4	IL-2 [‡]	IL-4 [§]	IL-10 [¶]
Term TNT	16	197 ± 67	879 ± 137	0.22	72 ± 14	1011 ± 176	285 ± 51
Term TT	7	411 ± 181	435 ± 96	0.94	289 ± 42	323 ± 34	32 ± 19
Normal	4	144 ± 42	791 ± 38	0.18	31 ± 9	851 ± 121	437 ± 152

*TT compared to TNT or N, $P < 0.01$.[†]TNT or N compared to TT, $P < 0.02$.[‡]TT compared to TNT, $P < 0.05$, TT compared to N, $P < 0.02$.[§]TNT compared to TT, $P < 0.05$, TNT not statistically different compared to N.[¶]TNT compared to TT, $P < 0.02$, TNT not statistically different compared to N.

Numbers represent mean ± SEM.

polymerase, directly to 200 ng of total RNA in 5 μ l of RNase, DNase-free water (Ambion, Austin, TX). Input RNA was normalized using glyceraldehyde-3-phosphate dehydrogenase mRNA quantification (PE Applied Biosystems). Reverse transcription and thermal amplification were performed using the following linked profile: reverse transcription 30 minutes at 60°C, cDNA denaturation 5 minutes at 95°C, and 40 cycles of denaturation (95°C for 15 seconds) and annealing/extension (60°C for 1 minute) in a 7700 sequence detection system (PE Applied Biosystems). Duplicate standard curves with copy number controls ranging from 10 copies to 10⁵ copies were run with each optical 96-well plate (PE Applied Biosystems). In addition, no template controls were included with each plate. Primer and probe sequences have been previously described.²⁰

Immunophenotyping/Fluorescence in Situ Hybridization

Tissue frozen in OCT embedding compound (Fisher Scientific, Pittsburgh, PA) were cut to 5 μ m and adhered to silanized slides (PE Applied Biosystems) followed by simultaneous immunophenotyping/fluorescence *in situ* hybridization as previously described.²⁴ Briefly, the sections were air-dried, rehydrated in PBS, and labeled with optimized concentrations of phycoerythrin-conjugated antibodies specific for the cell type of interest (CCR5, CXCR4) (PharMingen, San Diego, CA). HIV-1 mRNA was detected in the tissues using the ViroTect In Cell HIV-1 Detection System (Invirion, Frankfort, MI). The probe was hybridized to the target sequence for 60 minutes at 43°C, in a GeneAmp 1000 slide cycler (PE Applied Biosystems). Multiparameter analysis of cell surface receptors and HIV *gag-pol* mRNA was performed on a laser confocal microscope (Olympus, Melville, NY). Quantification of viral copies was performed using Metamorph software and fluorescein equivalents bead standards (Flow Cytometry Standards, San Juan, PR) and the formula:

$$\frac{\text{Total pixels (HIV}^+ \text{ cell-background)}}{\text{cell}} \times \frac{\text{FITC equivalents}}{\text{pixel}} \times \frac{0.007 \text{ copies}^*}{\text{FITC equivalent}} = \frac{\text{HIV-1 copies}}{\text{cell}}$$

*Based on 142 fluorescein equivalents per HIV-1 copy.

Statistical Analysis

Comparisons between transmitting and nontransmitting placentae were performed using either the Mann-Whitney rank sum test or paired *t*-test. Comparisons yielding a $P < 0.05$ were considered significant.

Results

Chemokine Receptor Expression in the Placenta

Total placenta RNA was extracted from 16 TNT and seven TT as well as four normal controls (Table 1). Real time reverse transcriptase-polymerase chain reaction was used for quantification of chemokine receptor expression. The CCR5:CXCR4 ratio was significantly increased in the TT placenta compared to TNT and normal controls. The number of CCR5 mRNA copies increased two- to threefold ($P < 0.01$) and the number of CXCR4 mRNA copies were reduced by half in the TT placenta compared to TNT and normal controls ($P < 0.02$, Table 1).

CXCR4 and CCR5 protein-expressing cells were analyzed at the single-cell level by *in situ* imaging in sections stained by immunohistochemistry (Figure 1, A and B). Quantification by *in situ* imaging showed that CXCR4 protein-expressing cells were present in equivalent numbers in TNT placenta compared to TT placenta. Conversely, CCR5 protein expression was up-regulated threefold in TT placenta compared to TNT placenta ($P < 0.02$). Chemokine receptor-positive cells were confined to placental macrophages and lymphocytes on the fetal side of the placenta villous (Figure 1A). Trophoblasts did not express either CXCR4 or CCR5 protein.

Characterization of the Cytokine Milieu in Placenta

It has been previously reported that type 1 and type 2 cytokines are involved in the regulation of chemokine receptor expression.²¹ To determine whether the chemokine receptor expression pattern observed in the placenta was associated with locally produced cytokines,

Figure 1. Representative quantification and localization of CCR5, CXCR4, and cytokine protein expression in placentae from transmitting and nontransmitting women using immunohistochemistry (A) and assisted computerized image analysis (B). Cells staining with a brown precipitate express the protein indicated (arrows). Cells were counterstained blue with hematoxylin. Chemokine receptor protein was expressed in placental lymphocytes and macrophages (Hofbauer cells) but not in trophoblasts. Scale bar, 20 μ m.

real-time reverse transcriptase-polymerase chain reaction gene quantification and *in situ* imaging were used to quantify cytokines in placenta tissue. In TNT placentae, there was a significant elevation of type 2 cytokine (IL-4 and IL-10) mRNA relative to type 1 (IL-2) mRNA expression ($P < 0.02$, Table 1). This cytokine pattern with a dominance of type 2 over type 1 cytokines was also observed in normal controls. The cytokine pattern observed in TT placentae was, on the other hand, reversed with an up-regulation of IL-2 and a down-regulation of type 2 cytokines compared to TNT placentae or normal control (all $P < 0.05$).

To confirm translation of cytokine mRNA into protein, tissue sections were stained by an immunohistochemistry technique and the local intracellular expression of cytokines was measured by *in situ* imaging. Type 2 cytokine (IL-4 and IL-10)-expressing cells were significantly up-regulated in TNT placentae compared to TT placentae ($P < 0.02$) (Figure 1, A and B). Biopsies from TT placentae showed significantly higher frequency of IL-2-expressing cells compared to TNT placentae ($P < 0.05$; Figure 1, A and B).

Chemokine Receptor Expression of Placental Cells Productively Infected by HIV-1

A sensitive *in situ* hybridization technique was used simultaneously with immunophenotyping to confirm selective utilization of chemokine receptors by HIV-1 in infected tissues.²¹ The transmitting placenta had a statistically significant increase in the number of *gag-pol* mRNA expressing cells as determined by quantitative laser confocal microscopy (2.3% versus 0.02%, $P < 0.001$) (Figure 2). More than 99% of cells expressing *gag-pol* mRNA were CCR5-positive cells in TT placenta samples. Conversely, a minority (<10%) of productively HIV-1-infected cells in the TNT placentae expressed CCR5 whereas the great majority of productively HIV-1-infected cells expressed CXCR4.

Based on a standard curve using fluorescein equivalent bead standards and the formula described in the Methods section, we calculated the number of viral copies per cell in TT and TNT placentae. Productively HIV-1-infected cells in the TT placentae averaged 214 viral copies per cell whereas productively HIV-1-infected cells in TNT placentae averaged 78 copies per cell demon-

Figure 2. Representative quantification and localization of cells expressing CCR5 or CXCR4 and HIV-1 *gag-pol* mRNA using UFISH with simultaneous CCR5 or CXCR4 immunofluorescence. Cells expressing CCR5 or CXCR4 alone appear red, cells expressing HIV-1 *gag-pol* mRNA appear green, and cells expressing both CCR5 or CXCR4 and HIV-1 *gag-pol* mRNA appear yellow-white depending on the amount of HIV-1 in the cell. The number of cells expressing HIV-1 *gag-pol* mRNA (green-white) and the number of HIV-1 copies per average infected cell were compared in TT (A), TNT (B), and normal placentae (C). Productively infected cells in TT placentae averaged 2.3% of mononuclear cells compared to 0.02% mononuclear cells in TNT placentae. Greater than 99% of productively infected cells in TT placentae were CCR5⁺ (A, arrowheads) whereas the majority of rare productively infected cells in TNT placentae were CXCR4⁺ (B, arrow). Productively infected cells in TT placentae average 214 HIV-1 copies per cell compared to 78 HIV-1 copies found in productively infected cells from TNT placentae suggesting an inhibition of HIV-1 replication in the TNT placentae. Dotted circles represent placental villi. Percentages and viral counts were based on the scanning of 10 fields with a surface area of 125.6 mm² each.

strating quantitative differences in HIV-1 production as well as tropism differences in TNT placentae compared to TT placentae.

Discussion

Numerous studies comparing HIV-1 gene sequences of mother-child-paired peripheral blood samples have described transmission of a homogeneous subset of maternal isolates.^{16–19} Although these types of studies continue to shed additional light on the nature of vertically transmitted HIV-1 isolates; little has been published examining the selective pressures in the placenta or birth canal that may influence transmission. Many factors have been proposed that influence maternal-fetal transmission. Factors associated with vertical transmission can be divided into maternal factors such as maternal viral load,^{25–27} maternal neutralizing antibody,^{28–31} or maternal HIV-1-specific cytotoxic T cell activity;³² maternal-placenta interface factors such as FasL expression^{33–35} or tumor necrosis factor-related apoptosis-inducing ligand/Apo-2L expression;³⁶ placental factors such as chorioamnionitis^{37,38} or HIV-suppressive activity;^{39,40} or fetal factors such as neutralizing antibodies or HIV-specific cytotoxic T cell.^{41–43} Here we present data to support an additional placental factor, that is, a type 2 to type 1 placental cytokine milieu shift drives chemokine receptor expression that selects for isolates demonstrated in numerous sequencing studies to be more likely transmitted.

Cytokines have been shown to increase or decrease HIV replication.⁴⁴ Previous studies of cytokine expression in the placenta have revealed production of a variety of type 1, type 2, and proinflammatory cytokines.^{45–52} The cytokine milieu of the placenta and the hormonal-cytokine network at the maternal-fetal interface has a crucial role in preventing fetal allograft rejection and supporting implantation. Allograft rejection is mediated by type 1 cytokines including interferon- γ and tumor necrosis factor- β .⁵² Several studies support the hypothesis that production of type 2 cytokines (IL-4, IL-10) may permit allograft tolerance and maintenance of pregnancy.^{45,46} Factors promoting a shift from a protolerance to a prorejection milieu remain to be fully characterized but may include infection, trauma, and vascular compromise. Evidence that HIV infection may create a prorejection milieu is supported by the increased risk of spontaneous abortion in HIV-infected women.⁵³ Our data suggest that placentae from nontransmitting women maintain a normal type 2 placental cytokine milieu whereas transmitting women have placentae that express type 1 cytokines. The mechanism underlying a shift from a type 2 cytokine dominance to a type 1 cytokine dominance in TT placentae is not clear although TT placentae tended to exhibit more chorioamnionitis or villitis. Because the type 1 cytokine milieu in placentae from transmitting women does not result in spontaneous abortion, the type 2 to type 1 shift in cytokine expression is likely to be a late event. This

is consistent with transmission during the third trimester and supports the efficacy of third trimester antiretroviral therapy.

The CCR5 chemokine receptor is required by nonsyncytium-inducing HIV-1 strains to infect target cells. Nonsyncytium-inducing isolates are the predominantly transmitted isolates from mother to infant. The role of CCR5 in vertical transmission has been predominantly studied in the context of genotype.^{54,55} These reports indicated that the presence of the homozygous $\Delta 32$ or heterozygous genotype among children of HIV-infected mothers has a protective role in transmission of HIV-1. For the first time, we here describe the role of CCR5 expression levels in selection for vertical transmission. Furthermore, we demonstrate that the type 2 cytokine milieu that predominates in normal full-term pregnancies and placentae from nontransmitting HIV-seropositive mothers drives the expression of CXCR4 resulting in a normally low CCR5:CXCR4 ratio in placental tissues. In addition, preferential up-regulation of CXCR4 mRNA by IL-10 occurs in *in vitro* cultures of placental-derived macrophages (B. K. Patterson, et al unpublished data). The observed up-regulation of CXCR4 could also be mediated by progesterone which was shown in a previous study by our laboratory to preferentially up-regulate CXCR4 production in peripheral blood mononuclear cells.²⁰ Taken together, these data strongly support the hypothesis that the normal placental milieu selects for isolates of HIV-1 less likely to be transmitted and, conversely, placental pathology selects for isolates that preferentially replicate in CCR5-expressing cells and consequently are more likely to be transmitted.

In situ imaging demonstrated that CCR5 and CXCR4 are expressed on placental macrophages and lymphocytes but not in trophoblasts. Simultaneous immunofluorescence *in situ* hybridization for HIV-1 *gag-pol* mRNA revealed that HIV-1 almost exclusively infects HIV-1 CCR5-expressing cells in placentae from transmitting women whereas HIV-1 infects predominantly CXCR4-expressing cells in nontransmitting placentae. A statistically significant increase in the total number of productively infected cells and in the number of viral particles produced per cell was also demonstrated in TT placentae compared to TNT placentae. These data suggest that the type 2 cytokine milieu and concomitant low CCR5:CXCR4 ratio may prevent HIV-1 replication in the placenta although the opposite is found in peripheral blood mononuclear cells. Conceivably, the normal placental cytokine environment may up-regulate β -HCG, a known inhibitor, or recently identified inhibitors of HIV-1 replication (B. K. Patterson, unpublished data). Our data, however, directly support previous studies that indicate vertical transmission of a homogeneous subset of maternal HIV-1 isolates that are nonsyncytium-inducing and use CCR5 as co-receptor.

In summary, our results indicate that immune-based interventions aimed at ways to augment the placental ability to prevent transmission may provide cost effective prevention strategies accessible to populations without access to drug therapy.

References

- European Collaborative Study: Risk factors for mother-to-child transmission of HIV-1. *Lancet* 1992, 339:1007-1012
- Ryder RW, Nsa W, Hassig SE, Behets F, Rayfield M, Ekungola B, Nelson AM, Mulenda U, Francis H, Mwandagaliwa K, Davachi F, Rogers M, Nzilambi N, Greenberg A, Mann J, Quinn TC, Piot P, Curran JW: Perinatal transmission of the human immunodeficiency virus type 1 to infants of seropositive women in Zaire. *N Engl J Med* 1989, 320:1637-1642
- Conner EM, Sperling RS, Gelber R, Kiselev P, Scott G, O'Sullivan MJ, VanDyke R, Bey M, Shearer W, Jacobson RL, Jimenez E, O'Neill E, Bazin B, Delfraissy J-F, Culnan M, Coombs R, Elkins M, Moya J, Stratton P, Balseley J: Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. *N Engl J Med* 1994, 331:1173-1180
- The International Perinatal HIV Group: The mode of delivery and the risk of vertical transmission of human immunodeficiency virus type 1—a meta-analysis of 15 prospective cohort studies. *N Engl J Med* 1999, 340:977-987
- Douglas GC, Fry GN, Thirkill T, Holmes E, Hakim H, Jennings M, King BF: Cell-mediated infection of human placental trophoblast with HIV in vitro. *AIDS Res Hum Retroviruses* 1991, 7:735-740
- David FJ, Autran B, Tran HC, Menu E, Raphael M, Debre P, Hsi BL, Wegman TG, Barre-Sinoussi F, Chouat G: Human trophoblast cells express CD4 and are permissive for productive infection with HIV-1. *Clin Exp Immunol* 1992, 88:10-16
- Toth FD, Mosborg-Petersen P, Kiss J, Aboagye-Mathiesen G, Zdravkovic M, Hager H, Aranyosi J, Lampe L, Ebbesen P: Antibody-dependent enhancement of HIV-1 infection in human term syncytiotrophoblast cells cultured in vitro. *Clin Exp Immunol* 1994, 96:389-394
- Lairmore MD, Cuthbert PS, Utley LL, Morgan CJ, Dezzutti CS, Anderson CL, Sedmak DD: Cellular localization of CD4 in the human placenta. Implications for maternal-to-fetal transmission of HIV. *J Immunol* 1993, 151:1673-1681
- Kilani RT, Chang LJ, Garcia-Lloret MI, Hemmings D, Winkler-Lowen B, Guilbert LJ: Placental trophoblasts resist infection by multiple human immunodeficiency virus (HIV) type 1 variants even with cytomegalovirus coinfection but support HIV replication after provirus transfection. *J Virol* 1997, 71:6359-6372
- Kristoffersen EK, Matre R: Co-localization of the neonatal Fc gamma receptor and IgG in human placental term syncytiotrophoblasts. *Eur J Immunol* 1996, 26:1668-1671
- Leach JL, Sedmak DD, Osborne JM, Rahill B, Lairmore MD, Anderson CL: Isolation from human placenta of the IgG transporter, FcRn, and localization to the syncytiotrophoblast: implications for maternal-fetal antibody transport. *J Immunol* 1996, 157:3317-3322
- Simister NE, Story CM, Chen HL, Hunt JS: An IgG-transporting Fc receptor expressed in the syncytiotrophoblast of human placenta. *Eur J Immunol* 1996, 26:1527-1531
- Spetz A-L, Patterson BK, Lore K, Andersson J, Holmgren L: Functional gene transfer of HIV DNA by an HIV receptor-independent mechanism. *J Immunol* 1999, 163:736-742
- Lewis SH, Reynolds-Kohler C, Fox HE, Nelson JA: HIV-1 in trophoblastic and villous Hofbauer cells, and hematological precursors in eight-week fetuses. *Lancet* 1990, 335:565-568
- McGann KA, Collman R, Kolson DL, Gonzalez-Scarano F, Coukos G, Coutifaris C, Strauss JF, Nathanson N: Human immunodeficiency virus type 1 causes productive infection of macrophages in primary placental cell cultures. *J Infect Dis* 1994, 169:746-753
- Briant L, Wade CM, Puel J, Leigh Brown AJ, Guyader M: Analysis of envelope sequence variants suggests multiple mechanisms of mother-to-child transmission of human immunodeficiency virus type 1. 1995, 69:3778-3788
- Menu E, Mbopi KF, Lagaye S, Pissard S, Maucelere P, Scarlatti G, Martin J, Goossens M, Chouat G, Barre-Sinoussi F: Selection of maternal human immunodeficiency virus type 1 variants in human placenta. European Network for In Utero Transmission of HIV-1. *J Infect Dis* 1999, 179:44-51
- Scarlatti G, Albert J, Rossi P, Hodara V, Biraghi P, Muggiasca L, Fenyo EM: Mother-to-child transmission of human immunodeficiency virus type 1: correlation with neutralizing antibodies against primary isolates. *J Infect Dis* 1993, 168:207-210
- Lamers SL, Sleasman JW, She JX, Barrie KA, Pomeroy SM, Barrett DJ, Goodenow MM: Persistence of multiple maternal genotypes of human immunodeficiency virus type 1 in infants infected by vertical transmission. *J Clin Invest* 1994, 93:380-390
- Patterson BK, Landay A, Andersson J, Brown C, Behbahani H, Jiyamapa D, Burki Z, Stanislawski D, Czerniewski MA, Garcia PM: Repertoire of chemokine receptor expression in the female genital tract: implications for human immunodeficiency virus transmission. *Am J Pathol* 1998, 153:481-490
- Patterson BK, Czerniewski MA, Andersson J, Sullivan Y, Su F, Jiyamapa D, Burki Z, Landay A: Regulation of CCR5 and CXCR4 expression by type 1 and type 2 cytokines: CCR5 expression is down-regulated by IL-10 in CD4-positive lymphocytes. *Clin Immunol* 1999, 91:254-262
- Andersson J, Fehniger TE, Patterson BK, Pottage J, Agnoli M, Jones P, Behbahani H, Landay A: Early reduction of immune activation in lymphoid tissue following highly active HIV therapy. *AIDS* 1998, 12: F123-F129
- Andersson U, Litton MJ, Fehniger TE, Ulfgren A-K, Andersson J: Detection and quantification of cytokine-producing cells by immunostaining. *Techniques in Quantification and Localization of Gene Expression*. Edited by BK Patterson. Boston, Birkhauser, 1999, pp 55-80
- Collins KB, Patterson BK, Naus GJ, Landers DV, Gupta P: Development of a novel in vitro organ culture model to study HIV-1 transmission in the female genital tract. *Nat Med* 2000, 6:475-479
- Garcia PM, Kalish LA, Pitt J, Minkoff H, Quinn TC, Burchett KC, Kornegay J, Jackson B, Moya J, Hanson C, Zorilla C, Lew JF: Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. *N Engl J Med* 1999, 341:394-402
- Shaffer N, Roongpisuthipong A, Siriwasin W, Chotpitayasunondh T, Cheerskul S, Young NL, Parekh B, Mock PA, Bhadrakom C, Chinayon P, Kalish ML, Phillips SK, Granade TC, Subbarao S, Weniger BG, Mastro TD: Maternal virus load and perinatal human immunodeficiency virus type 1 subtype E transmission, Thailand. Bangkok Collaborative Perinatal HIV Transmission Study Group. *J Infect Dis* 1999, 179:590-599
- Weiser B, Nachman S, Tropper P, Viscosi KH, Grimson R, Baxter G, Fang G, Reyelt C, Hutcheon N, Burger H: Quantitation of human immunodeficiency virus type 1 during pregnancy: relationship of viral titer to mother-to-child transmission and stability of viral load. *Proc Natl Acad Sci USA* 1994, 91:8037-8041
- Ugen KE, Srikantan V, Goedert JJ, Nelson Jr RP, Williams WV, Weiner DB: Vertical transmission of human immunodeficiency virus type 1: seroreactivity by maternal antibodies to the carboxy region of the gp41 envelope glycoprotein. *J Infect Dis* 1997, 175:63-69
- St Louis ME, Kamenga M, Brown C, Nelson AM, Manzila T, Batter V, Behets F, Kabagabo U, Ryder RW, Oxtoby M: Risk for perinatal HIV-1 transmission according to maternal immunologic, virologic, and placental factors. *JAMA* 1993, 269:2853-2859
- Khoury YF, McIntosh K, Cavacini L, Posner M, Pagano M, Tuomala R, Marasco WA: Vertical transmission of HIV-1. Correlation with maternal viral load and plasma levels of CD4 binding site anti-gp120 antibodies. *J Clin Invest* 1995, 95:732-737
- Fenyo EM, Putkonen P: Broad cross-neutralizing activity in serum is associated with slow progression and low risk of transmission in primate lentivirus infections. *Immunol Lett* 1996, 51:95-99
- Jin XC, Roberts G, Nixon DF, Cao Y, Ho DD, Walker BD, Muldoon M, Korber BT, Koup RA: Longitudinal and cross-sectional analysis of cytotoxic T lymphocyte responses and their relationship to vertical human immunodeficiency virus transmission. *J Infect Dis* 1998, 178: 1317-1326
- Hunt JS, Vassmer D, Ferguson TA, Miller L: Fas ligand is positioned in mouse uterus and placenta to prevent trafficking of activated leukocytes between the mother and the conceptus. *J Immunol* 1997, 158:4122-4128
- Menu E, Kaplan L, Andreu G, Denver L, Chouat G: Immunoactive products of human placenta. I. An immunoregulatory factor obtained from explant cultures of human placenta inhibits CTL generation and cytotoxic effector activity. *Cell Immunol* 1989, 119:341-352
- Uckan D, Steele A, Cherry, Wang BY, Chamizo W, Koutsoukoulis A, Gilbert-Barnes E, Good RA: Trophoblasts express Fas ligand: a proposed mechanism for immune privilege in placenta and maternal invasion. *Mol Hum Reprod* 1997, 3:655-662
- Phillips TA, Ni J, Pan G, Ruben SM, Wei Y-F, Pace JL, Hunt JS: TRAIL

- (Apo-2L) and TRAIL receptors in human placentae: implications for immune privilege. *J Immunol* 1999, 162:6053–6059
37. Ladner J, Leroy V, Hoffman P, Nyiraziraje M, De Clercq A, Van D, Perre P, Dabis F: Chorioamnionitis and pregnancy outcome in HIV-infected African women. Pregnancy and HIV Study Group. *J AIDS Hum Retrovirol* 1998, 18:293–298
 38. Wabwire-Mangen F, Gray RH, Mmiro FA, Ndugwa C, Abramowsky C, Wabinga H, Whalen C, Li C, Saah AJ: Placental membrane inflammation and risks of maternal-to-child transmission of HIV-1 in Uganda. *J Acquired Immune Defic Syndr* 1999, 22:379–385
 39. Lunardi-Iskandar Y, Bryant JL, Blattner WA, Hung CL, Flamand L, Gill P, Hermans P, Birken S, Gallo RC: Effects of a urinary factor from women in early pregnancy on HIV-1, SIV and associated disease. *Nat Med* 1998, 4:428–434
 40. Bourinbaier AS, Lee-Huang S: Anti-HIV effect of beta subunit of human chorionic gonadotropin (β -HCG) in vitro. *Immunol Lett* 1995, 44:3–18
 41. Jenkins M, Landers D, Williams-Herman D, Wara D, Viscarello RR, Hamill HA, Kline MW, Shearer WT, Charlebois ED, Kohl S: Association between anti-human immunodeficiency virus type 1 (HIV-1) antibody-dependent cellular cytotoxicity antibody titers at birth and vertical transmission of HIV-1. *J Infect Dis* 1994, 170:308–312
 42. Luzuriaga K, McQuilken P, Alimenti A, Somasundaran M, Hesselton R, Sullivan JL: Early viremia and immune responses in vertical human immunodeficiency virus type 1 infection. *J Infect Dis* 1993, 167:1008–1013
 43. Pikora CA, Sullivan JL, Panicali D, Luzuriaga K: Early HIV-1 envelope-specific cytotoxic T lymphocyte responses in vertically infected infants. *J Exp Med* 1997, 185:1153–1161
 44. Poli G, Fauci AS: Cytokine Cascades in HIV Infection. Edited by S Gupta. New York, Plenum Press, 1996
 45. Chaouat G, Menu E, de Smedt D, Khrihnan L, Hui L, Assal MA, Martal J, Raghupathy R, Wegmann TG: The emerging role of IL-10 in pregnancy. *Am J Reprod Immunol* 1996, 35:325–329
 46. Deneys V, De Bruyere M: Immunological tolerance of fetal allograft: efficacy of immunotherapy and IL-4 and TNF alpha serum levels in recurrent abortion. *Transplant Proc* 1997, 29:2467–2469
 47. Lee BN, Ordonez N, Popek EJ, Lu JG, Helfgott A, Eriksen N, Hamill HA, Kozinetz C, Doyle M, Kline M, Langston C, Shearer WT, Reuben JM: Inflammatory cytokine expression is correlated with the level of human immunodeficiency virus (HIV) transcripts in HIV-infected placental trophoblastic cells. *J Virol* 1997, 71:3628–3635
 48. Marzi M, Vigano A, Trabattini D, Villa ML, Salvaggio A, Clerici E, Clerici M: Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. *Clin Exp Immunol* 1996, 106:127–133
 49. Piccinni M-P, Giudizi M-G, Biagiotti R, Beloni L, Giannarini L, Sampognaro S, Parronchi P, Manetti R, Annunziato F, Livi C, Romagnani S, Maggi E: Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. *J Immunol* 1995, 155:128–133
 50. Piccinni MP, Beloni L, Livi C, Maggi E, Scarselli G, Romagnani S: Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. *Nat Med* 1998, 4:1020–1024
 51. Reuben JM, Turpin JA, Lee BN, Doyle M, Gonik B, Jacobson R, Shearer WT: Induction of inflammatory cytokines in placental monocytes of gravidae infected with the human immunodeficiency virus type 1. *J Interferon Cytokine Res* 1996, 16:963–971
 52. Wegmann TG, Lin H, Guilbert L, Mossman TR: Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol Today* 1990, 14:353–356
 53. Langston C, Lewis DE, Hamill HA, Popek EJ, Kozinetz CA, Kline MW, Hanson IC, Shearer WT: Excess intrauterine fetal demise associated with maternal human immunodeficiency virus infection. *J Infect Dis* 1995, 172:1451–1460
 54. Mas A, Espanol T, Heredia A, Pedraza MA, Hernandez M, Caragol I, Fernando M, Bertran JM, Alcamí J, Soriano V: CCR5 genotype and HIV-1 infection in perinatally-exposed infants. *J Infect Dis* 1999, 38: 9–11
 55. Philpott S, Burger H, Charbonneau T, Grimson R, Vermund SH, Visosky A, Nachman S, Kovacs A, Tropper P, Frey H, Weiser B: CCR5 genotype and resistance to vertical transmission of HIV-1. *J Acquired Immune Defic Syndr* 1999, 21:189–193