THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PH.D.)

IN VITRO INVESTIGATION OF THE ROLE OF PLACENTAL MACROPHAGES IN THE VERTICAL TRANSMISSION OF HUMAN CYTOMEGALOVIRUS AND HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

ATTILA BÁCSI

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ATTILA BÁCSI

SUPERVISOR: PROF. FERENC D. TÓTH

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INTRODUCTON

Intrauterine infection of the fetus is clearly an important mode of vertical transmission of human cytomegalovirus (HCMV) and human immunodeficiency virus type 1 (HIV-1). The exact mechanism of the transplacental transmission of the viruses is unknown.

The placental syncytiotrophoblast (ST) forms a continuous, multinucleated epithelium in direct contact with maternal blood and constitutes the critical selective barrier between maternal and fetal tissues. Thus, syncytiotrophoblast must be traversed by viruses in order to reach underlying cytotrophoblastic cells, placental macrophages (Hofbauer cells), fibroblasts, and endothelial cells lining the fetal capillaries.

Transmission of HIV-1 through the placenta could involve transport of infected cells across the syncytiotrophoblast barrier or infection of syncytiotrophoblast cells. The question of the infection of syncytiotrophoblast cells remains controversial. Even if the presence of proviral DNA of HIV-1 has been detected in the syncytiotrophoblast layer in situ, *in vitro* studies are more conflicting. Several studies have demonstrated that cultured human trophoblasts are moderately, but effectively, susceptible to infection by laboratory strains of HIV-1. In contrast, other investigators have been unable to demonstrate any trophoblast infection with cell-free virus and claim that cell-cell contact is an absolute requirement for HIV-1 infection of syncytiotrophoblast cells.

Conflicting results may be due to several experimental variables, such as differences in HIV-1 isolates used for infection of syncytiotrophoblast cells or the use of different detection assays. The phenotypic mixing between HIV-1 and vesicular stomatitis virus (VSV) has been exploited to assay the susceptibility of syncytiotrophoblast cells to penetration by various strains of HIV-1. When cells infected with HIV-1 are superinfected with VSV, a proportion of the progeny VSV assemble sufficient envelope glycoprotein of the retrovirus to resist neutralization by hyperimmune anti-VSV serum. The host range of such pseudotype virions is restricted to cells expressing receptors specific to the HIV-1. Following penetration of the cell and uncoating of the virus, the transcapsidated VSV genome replicates to produce non-pseudotype particles which destroy the cells. Thus, VSV(HIV-1) pseudotypes provide a simple cytopathic assay for studying events in HIV-1 replication cycle that are determined by viral envelope properties.

It has been showed by in situ immunohistochemistry and *in vitro* studies, that ST cells can be infected by HCMV. Although it remains unclarified, how the virus can cross the ST layer. Previous studies have indicated that ST cells do not support the complete viral

reproductive cycle or HCMV replication may occur in a small portion of ST cells. Investigators usually can detect only immediate-early proteins of HCMV in infected ST cells.

In syncytiotrophoblast cultures infected with HCMV or HIV-1, infectious virus could not be detected in supernatants, or the rate of virus production is very low. These findings suggest that productive infection of trophoblasts occurs only in limited circumstances and several cofactors are likely involved in this process.

The role of adhesive interactions between placental macrophages and ST cells in vertical transmission of HCMV and HIV-1 has not been studied. These interactions may be of particular importance, because, together with fibroblasts, macrophages constitute the majority of connective tissue cells in human placenta. Furthermore, both *in vitro* and *in vivo* studies demonstrated that placental macrophages can be permissively infected with HCMV and HIV-1, respectively. Hence, macrophages may act as a vector, delivering the virus to the placental tissue and villous capillaries.

Recent studies have shown that direct cell-to-cell interaction is able to reactivate both HCMV and HIV-1 from latency. The present study tested the possibility that placental macrophages might enhance activation of HCMV or HIV-1 carried in ST cells, and further, that infected ST cells would be capable of transmitting virus to neighbouring macrophages. Since direct cell-cell contact can induce the synthesis of various cytokines, we also hypothesized that cytokine production by cocultured cells may have a key role in upregulation of HCMV and HIV-1 gene expression. For this purpose, we investigated whether there is an effect of cocultivation on cytokine production of syncytiotrophoblasts and placental macrophages. If yes, how it can influence the HCMV and the HIV-1 replication.

Interestingly enough, from epidemiological studies, the in utero transmission of HIV-1 seems to occur either late in pregnancy or in early pregnancy. Several studies reported indirect evidence that late in utero infection accounts for the vast majority of transplacental transmission of HIV-1. Since HCMV crosses the placenta at all stages of gestation, the time of primary infection in pregnancy is not associated with an increased or decreased risk of vertical transmission.

On the basis of the above findings, we have separated trophoblasts and macrophages from human term placentas and examined the susceptibility of ST cells to HCMV or HIV-1 infection either alone or in coculture with placental macrophages.

MATERIALS AND METHODS

Viruses

The AD169 strain of HCMV was propagated on human embryonal fibroblast cultures. Supernatants were harvested from infected cells and HCMV was then pelleted by ultracentrifugation. The pellet was resuspended in 1 ml of medium for each 30 ml of supernatant. VSV, Indiana strain, was maintained routinely and titrated by plaque-forming unit (PFU) assay on WISH cells. The IIIB, RF and MN strains of HIV-1 were grown in H9 cells. The Ba-L and Ada-M strains of HIV-1 were propagated in monocyte-derived macrophage cultures. For some experiments concentrated stocks of Ba-L strain of HIV-1 were preparated by ultracentrifugation.

HCMV titration

Viral stocks were titrated on human embryonal fibroblasts by standard plaque assay. The titer of HCMV was determined by the numbers of cytopathic plaques in different dilutions of the sample and expressed in PFU/ml.

HIV titration

Depending on the type of experiments HIV production was assayed in three ways: by measuring the reverse transcriptase activity of the virus, by enzyme-linked immunosorbent assay (ELISA) for extracellular p24 core antigen and syncytium-forming assay as a measure of infectious virus.

Isolation and culture of cytotrophoblast cells and macrophages (Hofbauer cell)

Trophoblasts and macrophages were separated in parallel from human term placental samples. The isolation of pure villous cytotrophoblasts from placenta was performed by using a discontinuous Percoll gradient and the remaining contaminating cells were then removed by immunomagnetic micropheres. For in vitro differentiation of trophoblast cells KGM (keratinocyte growth medium) supplemented with 2 mM glutamine, antibiotics and 15% fetal calf serum (FCS) was used. We used 5-day-old ST cultures consisting of multinucleated differentiated cells in all experiments.

Placental macrophages were isolated by centrifugation over Ficoll-Hypaque and Percoll gradient. The cells were cultured in RPMI 1640 medium containing 2 mM glutamine, 10% human serum (HuSe), and antibiotics.

Isolation and growth of monocyte-derived macrophages

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation from buffy coat preparations. Mononuclear cells were cultured in RPMI-1640 medium with 2 mM glutamine, antibiotics, and 10% pooled HuSe. In our experiments 7-day-old macrophage cultures were infected with viruses.

Cell lines

The epithelium-derived WISH and MDBK cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% FCS. The H9, Sup-T1, CEM-SS and U937 cells were maintained in RPMI-1640 medium containing 10% FCS. Media also contained 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. All cell lines were free of mycoplasma contamination at the time of the experiments.

HCMV infection of ST cell cultures

The 5-day-old syncytiotrophoblast cultures were infected with HCMV at a multiplicity of infection of 1.0 PFU/cell, calculated according to the number of cytotrophoblast cells seeded for syncytium formation. The cells were incubated with virus for 2 h at 37 °C.

Production of pseudotype viruses

Pseudotype virus was produced in a two-step procedure: HIV infection followed by superinfection with VSV. The promonocytic U937 cells were infected with IIIB, RF or MN strains of HIV-1 and Sup-T1 cells were infected with Ba-L and Ada-M strains of HIV-1 to become permanently infected virus producers. U937 or Sup-T1 cells chronically infected with HIV-1 were superinfected with VSV at a multiplicity of infection of 10 PFU/cell. The cultures were harvested 12-18 h later, clarified, and aliquots were frozen at -70^{o}C.

Assay of pseudotype viruses

Virus samples were incubated for 1 h at 37^{o}C with excess heat-inactivated rabbit anti-VSV serum to titrate the pseudotype or without antiserum to titrate the total VSV. Human anti-HIV-1 sera were included at appropriate dilutions at this stage when required. Pseudotypes

bearing the envelope glycoproteins of IIIB, RF or MN strains of HIV-1 were plated on monolayers of CEM-SS cells attached to the plastic using poly-L-lysine. For putative VSV(HIV-1) virus stocks pseudotyped with the envelope glycoproteins of Ba-L or Ada-M strains of HIV-1, primary macrophage cultures were used as target assay cells. Cells were incubated with VSV(HIV-1) pseudotypes and after virus adsorption the cells were washed and overlaid with excess bovine MDBK cells to act as plaque indicator cells for progeny VSV released from human cells infected with pseudotype. After allowing 2 h for attachment of MDBK cells, the cells were overlaid with agar medium. Plaques were counted 1-2 days after plating and expressed in PFU/ml.

Infection of syncytiotrophoblast cells with VSV and VSV(HIV-1) pseudotypes

Five-day-old syncytiotrophoblast cultures were infected with VSV or VSV(HIV-1) pseudotypes. Supernatants of virus-infected and uninfected control cultures were collected at different time intervals after infection and virus titers were determined by plaque assay on WISH cells. In order to test the plating efficiency of VSV and VSV(HIV-1) pseudotypes, syncytiotrophoblasts were washed and overlaid with MDBK cells after virus adsorption. After attachment of the MDBK cells (2 h), the cells were overlaid with agar medium. Plaque formation was evaluated 1-2 days postinfection.

HIV-1_{Ba-L} infection of ST cell cultures

The 5-day-old ST cultures were infected with HIV-1 at a multiplicity of infection of 1.0 syncytium-forming unit /cell, calculated according to the number of cytotrophoblast cells seeded for syncytium formation. The HIV-1 preparations were treated with DNase before their use for infection. The cells were incubated with virus for 2 h at 37^{o}C.

Coculture system

After detachment, macrophages were added to HCMV-infected or uninfected cultures of 9-day-old ST cells at a macrophage to trophoblast numerical ratio of 1.0.

Immune sera and conjugates

Mouse monoclonal antibodies (MAb) were used for recognition the HCMV immediate-early and lower matrix protein pp65 proteins, respectively. MAbs to CD4, CXCR4, CCR3 and CCR5 were tested for their ability to block VSV(HIV-1) infection of syncytiotrophoblasts. Human HIV immunoglobulin and HIV neutralizing serum were used in some experiments.

VSV infection was inhibited by hyperimmune rabbit anti-VSV antiserum. To identify HIV-1 infected cells, experiments were performed with MAb specifically reactive with the p24 core antigen of HIV-1, and rabbit antiserum raised against HIV-1 Tat protein. To identify the cytokine producing cells in cocultures mouse MAbs to human IL-1β, IL-6, IL-8, TGF-β1 and TNF-α were used.

Fluorescein isothiocyanate (FITC)-conjugated mouse MAb to CD68 macrophage-specific marker, rabbit anti-mouse immunoglobulin (Ig) conjugated to tetramethyl rhodamine isothiocyanate (TRITC), TRITC-conjugated swine anti-rabbit Ig, rabbit anti-mouse Ig conjugated to FITC, and swine anti-rabbit Ig conjugated to FITC were consumed in two-color immunofluorescence tests.

Immunofluorescence assays (IFA)

Cells expressing virus-specific proteins or cytokines were demonstrated by a combination of direct and indirect cytoplasmic immunofluorescence assay. In the first phase of the reaction, indirect cytoplasmic IFA was performed. In the second phase of the reaction, cells were incubated with FITC-conjugated MAb to CD68.

By the production of pseudotype viruses, the expression of HIV-1 p24 protein in persistently infected cells and the presence of VSV specific proteins in ST cells were tested with an indirect cytoplasmic IFA.

Assay for cytokine determination

The production of cytokines from uninfected and virus-infected cultures was determined by commercial ELISA kits for IL-8, TGF-β1, IL-1α, IL-1β, IL-6, GM-CSF, M-CSF, and TNF-α. Cytokine concentrations in supernatants were measured according to the manufacturer's guidelines.

RESULTS

I. Infectious virus could not be detected in supernatants of HCMV-infected ST cells throughout an 18-day period of observation. In contrast, coculture of virus-infected ST cells with placental macrophages resulted in production of infectious HCMV. As an indicator of HCMV infection with respect to viral entry and replication, cells were immunostained for expression of HCMV IE and pp65 antigens. The cell types in the cocultures were identified by using antibody to CD68 macrophage-specific marker. In HCMV-infected ST cells cultured alone, IE gene expression was detected as early as at day 1 after infection. In contrast, no pp65 protein could be detected in noncocultivated ST cells. In virus-infected ST cells cocultured with placental macrophages, expression of pp65 antigen was first detected on day 3 of coculturing. Dual immunofluorescence staining for macrophage marker CD68 and IE antigen demonstrated that IE antigen appeared in macrophages on day 6 of coculturing. On day 8 the pp65 antigen also was detectable in macrophages cocultured with HCMV-infected ST cells. Thus, HCMV-infected ST cells were capable of transmitting virus to cocultured macrophages.

To determine which of the cytokine-inducing activities of coculture may have a role in induction of viral replication, we investigated IL-8 and TGF-β1 secretion in uninfected, virus infected and cocultured cell systems. Uninfected ST cultures constitutively released low levels (0.8 ng/ml) of IL-8 into the culture medium. The mean value of IL-8 release from uninfected macrophages was about 2.4 ng/ml. In ST cultures infected with HCMV, a slight increase in IL-8 production was found compared with that in uninfected cell cultures. However, coculture of HCMV-infected ST cells with placental macrophages induced the secretion of large amounts of IL-8 into the culture supernatant in a time-dependent manner. The IL-8 production induced by coculture reached a peak value of 17.8 ng/ml on day 3 of coculturing and gradually decreased over the next 6 days. Surprisingly, coculture of uninfected ST cells with placental macrophages induced the same kinetics and magnitude of IL-8 secretion as coculture of virus-infected ST cells with placental macrophages. The rate of constitutive TGF-β1 production by uninfected ST cells and macrophages was 3.6 ng/ml and 2.8 ng/ml, respectively. No significant increase in TGF-β1 secretion by ST cells upon infection with HCMV was observed. In contrast, coculture of virus-infected ST cells with uninfected macrophages resulted in a rapid production of massive amounts of TGF-β1. Maximal TGF-β1 release (21.8 ng/ml) from cell cultures was observed on day 3 of

coculturing. Then the TGF-β1 production gradually decreased by day 9. It is of note that the amounts of TGF-β1 induced by coculture of uninfected ST cells with macrophages was comparable to those elicited by coculture of HCMV-infected ST cells with macrophages. To identify the cells producing substantial amounts of IL-8 and TGF-β1 in the coculture system, we performed double immunofluorescence staining. Macrophages adhering to ST cells were strongly stained with anti-IL-8 antibodies and anti-TGF-β1 antibodies, whereas faint fluorescence was noted in ST cells.

On the basis of the results of the foregoing experiments, we postulated that increased cytokine production may be mediator of coculture-induced HCMV replication. To address this question, neutralizing antibodies to IL-8 and TGF-β1 were used as means of blocking cytokine activities. Anti-cytokine antibodies were applied to the cells in 10-fold excess. Treatment of cells was started immediately after adding macrophages to HCMV-infected ST cultures and continued for another 10 days. Treatment with anti-IL-8 and anti-TGF-β1 antibody resulted in a 24-fold and 35-fold decrease in peak levels of HCMV release, respectively. It is noteworthy that the period of HCMV production by cocultures was also shortened due to anti-cytokine treatments. When anti-IL-8 and anti-TGF-β1 treatments were combined, no infectious virus was found in supernatants of cell cultures.

II. Pseudotyped VSV may be used to assay the ability of the virus strain which provides the envelope to penetrate various cell types, providing the genomic portion of the pseudotype is capable of replicating in that cell type. Thus, the ability of ST cells to be permissively infected with VSV was first determined. In cell cultures infected with VSV, release of infectious virus was detected. VSV caused rounding up and lysis of infected syncytiotrophoblast cells. In infected ST cultures VSV protein expression could be detected by indirect cytoplasmic IFA.

In the next phase of our experiments pseudotype viruses bearing the Env proteins of T-cell-tropic IIIB or MN, dual-tropic RF, macrophage-tropic Ada-M or Ba-L strains of HIV-1 were produced in U937 and Sup-T1 cells cultures. Next, the ability of VSV(HIV-1) pseudotypes to enter syncytiotrophoblast cells was investigated. Infectivities of VSV and the five VSV(HIV-1) pseudotypes were compared on the respective target cells and the syncytiotrophoblasts in the presence of excess anti-VSV serum. VSV(HIV-1_{RF}), VSV(HIV-1_{MN}) and VSV(HIV-1_{Ada-M}) pseudotypes failed to produce plaques above background on syncytiotrophoblast cells. VSV(HIV-1_{IIIB}) pseudotype plated on syncytiotrophoblast cells had

a 10-fold lower titer than on CEM-SS cells, but 100-fold higher than the background plaques. Similarly, the VSV(HIV-1_{Ba-L}) pseudotype had a 10-fold higher plating efficiency on macrophages than on syncytiotrophoblast cells. These data indicated that syncytiotrophoblast cells were susceptible to both VSV(HIV-1_{IIIB}) and VSV(HIV-1_{Ba-L}) pseudotypes but not to VSV pseudotyped with envelope glycoproteins of RF, MN or Ada-M strains of HIV-1.

To determine whether the infectivity of VSV(HIV-1_{IIIB}) and VSV(HIV-1_{Ba-L}) pseudotypes on syncytiotrophoblast cells was due to entry via HIV-1 envelopes, effect of anti-HIV-1 sera on virus production by syncytiotrophoblasts was studied. Addition of the appropriate neutralizing sera to the viral preparates substantially inhibited infection of syncytiotrophoblast cells with VSV(HIV-1) pseudotypes. The infectivity of VSV(HIV-1_{IIIB}) virus was also reduced by the human HIV immunoglobulin, albeit to a lesser degree than that of VSV(HIV-1_{Ba-L}) pseudotype. In contrast, the human HIV neutralizing serum failed to neutralize the VSV(HIV-1_{Ba-L}) pseudotype. These findings demonstrated that entry of VSV(HIV-1) pseudotypes into syncytiotrophoblast cells was mediated by Envs from IIIB and Ba-L strains of HIV-1.

MAb to CD4 was tested for its ability to block VSV(HIV-1) infection of syncytiotrophoblasts, by incubating the cells with antibody before plating the pseudotypes. Anti-CXCR4 MAb was used to test its ability to prevent VSV(HIV-1_{IIIB}) infection. The anti-CCR5 MAb was tested for its ability to inhibit infection with VSV(HIV-1_{Ba-L}). The ability of anti-CCR3 MAb to exert blocking effect upon infection with VSV(HIV-1_{IIIB}) or VSV(HIV-1_{Ba-L}) was also assessed. Blocking effect of the above antibodies on VSV(HIV-1) infection of macrophages and CEM-SS cells was studied in parallel experiments. MAbs to CXCR4, CCR5 and CCR3 were added to the cells either alone or in combination with anti-CD4 MAb. MAbs were tested for pseudotype inhibition by treating target cells before infection with VSV(HIV-1_{IIIB}) or VSV(HIV-1_{Ba-L}). As expected, infection of CEM-SS cells with VSV(HIV-1_{IIIB}) was blocked by both anti-CD4 and anti-CXCR4 MAbs. Antibodies to CD4 and CCR5 inhibited VSV(HIV-1_{Ba-L}) infection of macrophages. In agreement with previous observations, anti-CCR3 MAb did not affect the levels of progeny virus production by VSV(HIV-1_{IIIB})-infected CEM-SS cells or VSV(HIV-1_{Ba-L})-infected macrophages. Neither the anti-CD4 MAb nor the anti-CXCR4, anti-CCR5 and anti-CCR3 MAbs had any inhibitory effect upon infection of syncytiotrophoblast cells with VSV(HIV-1) pseudotypes. Pretreatment of syncytiotrophoblasts with anti-CD4 MAb in combination with anti-chemokine-receptor MAbs led to the same results.

III. The next study tested the possibility that placental macrophages might induce replication of HIV-1 carried in ST cells. Because macrophage-tropic HIV-1 strains are transmitted preferentially during pregnancy and our previous data showed that Ba-L strain of HIV-1 capable of entering ST cells, this strain was used throughout our studies. Virus release could not be detected in supernatants of HIV-1-infected ST cells throughout a 22-day period of observation. In contrast, coculture of virus-infected ST cells with placental macrophages resulted in HIV-1 production. Virus release was first detected on day 5 of coculturing. Concentrations of HIV-1 p24 in the supernatants of cocultivated cells reached a maximum value on day 16. In concert with HIV-1 antigen production, release of infectious virus reached a maximum value on day 16, after which the rate of HIV-1 production gradually decreased.

As an indicator of HIV-1 gene expression, infected cell cultures were immunostained for HIV-1 Tat and p24 antigens. In HIV-1-infected ST cells cultured alone no Tat or p24 protein were found. In virus-infected ST cells cocultured with placental macrophages, Tat protein was detected as early as 2 days of coculturing and peak levels of Tat protein expression were observed at 6-12 days. Expression of p24 antigen in HIV-1-infeced ST cells was first detected on day 4 of coculturing. The ratio of p24 antigen-positive cells reached a maximum value on day 8. Dual immunofluorescence staining for macrophage marker CD68 and Tat protein demonstrated that Tat protein appeared in macrophages on day 7 of coculturing. The proportion of cells expressing Tat protein then sharply increased, reaching a peak value of 81% at day 12. The p24 antigen was detectable first on day 9 in macrophages cocultured with HIV-1-infected ST cells, and the highest number of cells showed p24 antigen-specific fluorescence at days 14-16. Thus, HIV-1-infected ST cells were capable of transmitting virus to cocultured macrophages.

Because direct cell-cell contact can induce the synthesis of various cytokines, we hypothesized that cytokine production by cocultured cells may have a key role in upregulation of HIV-1 gene expression. We investigated IL-1α, IL-1β, IL-6, GM-CSF, M-CSF, and TNF-α secretion in uninfected, virus-infected, and cocultured systems. No measurable secretion of IL-1α, GM-CSF, and M-CSF was found in any cell culture.

Uninfected ST cells and placental macrophages constitutively released less than 20 pg/ml of IL-6 into the culture medium. Infection of ST cells with HIV-1 did not increase the rate of IL-6 production. However, coculture of uninfected ST cells with placental macrophages induced the secretion of large amounts of IL-6 into the culture supernatant. The IL-6 production induced by coculture reached a peak value of 5175 pg/ml on day 1 of

coculturing. Then IL-6 secretion decreased to control levels by day 6. Infection of ST cells with HIV-1 before coculture further increased the rate and longevity of IL-6 production induced by coculture of uninfected ST cells with placental macrophages. The secretion of IL-6 reached a second peak value of 7250 pg/ml on day 10. The same level of IL-6 production was maintained for the next four days. Then the IL-6 secretion declined.

The rate of constitutive TNF-α production by uninfected ST cells and macrophages was less than 10 pg/ml. No significant increase in TNF-α secretion by ST cells on infection with HIV-1 was observed. However, coculture of uninfected ST cells with placental macrophages resulted in a stimulation of TNF-α production. TNF-α release from cell cultures reached a maximum value of 480 pg/ml on day 1 and decreased thereafter. The TNF-α production was ceased by day 7. Coculture of virus-infected ST cells with placental macrophages led to a further increase in the rate and duration of TNF-α production compared with that in coculture of uninfected ST cells and macrophages. A second peak in TNF-α release (1250 pg/ml) was observed on day 10 of coculturing, after which the TNF-α production gradually decreased.

No spontaneous release of IL-1β was found in cultures of uninfected and HIV-1-infected ST cells. Placental macrophages cultured alone constitutively released low levels (8 pg/ml) of IL-1β into the medium. Interestingly, coculture of placental macrophages with uninfected ST cells did not result in elevated levels of IL-1β secretion. When placental macrophages were cocultured with HIV-1-infected ST cells, significant increase in IL-1β production was detected as compared with that in coculture of uninfected ST cells and macrophages. The increase in IL-1β secretion occurred on day 6 of coculturing and continued by day 10, reaching a maximal level of 265 pg/ml. Then there was a gradual decrease in IL-1β activity of supernatants.

To identify the cells producing substantial amounts of IL-1β, IL-6 and TNF-α in the coculture system, we performed double immunofluorescence staining. Cocultures of uninfected ST cells and placental macrophages were examined for IL-6 and TNF-α production at 24 h of coculturing. Macrophages adhering to ST cells for 1 day were strongly stained with anti-IL-6 antibodies, whereas faint fluorescence was noted in ST cells. Similarly, the anti-TNF-α antibodies were seen to react primarily with macrophages, and less intense fluorescence was seen in the ST cells. Cocultures of HIV-1-infected ST cells and placental macrophages were immunostained for IL-1β, IL-6 and TNF-α on day 10 of coculturing. The reactivity of anti-IL-1β MAb was predominantly in the macrophages, and faint fluorescence

was seen in the ST cells. Immunofluorescence staining performed with MAb to IL-6 and TNF-α gave essentially the same results.

On the basis of the results of the foregoing experiments, we postulated that increased cytokine production may be the mediator of coculture-induced HIV-1 replication. To address this question, neutralizing antibodies to IL-1β, IL-6 and TNF-α were used as a means of blocking cytokine activities. Anticytokine antibodies were applied to the cells in 10-fold excess. Treatment of cells was started immediately after adding macrophages to HIV-1-infected ST cultures and continued for another 6 days. Treatment with anti-TNF-α and anti-IL-6 antibody resulted in a 12-fold and 35-fold decrease in peak levels of HIV-1 release, respectively. When anti-TNF-α and anti-IL-6 treatments were combined, no HIV-1 production was found during 22 days of coculture. In contrast to anti-TNF-α and anti-IL-6, anti-IL-1β antibody had no inhibitory effect on coculture-induced HIV-1 replication. Similarly, the degree of inhibition exerted by anti-TNF-α or anti-IL-6 treatment could not be increased by combination with anti-IL-1β antibody.

SUMMARY

1. We studied HCMV replication in ST cells grown alone or cocultured with uninfected placental macrophages. Our results demonstrated that HCMV gene expression in ST cells was markedly upregulated by coculture with macrophages, resulting in release of substantial amounts of infectious virus from HCMV-infected ST cells. After having become permissive for viral replication, ST cells delivered HCMV to the cocultured macrophages as evidenced by detection of virus-specific antigens in these cells. The stimulatory effect of coculture on HCMV gene expression in ST cells was mediated by marked interleukin-8 and transforming growth factor-β1 release from macrophages, an effect caused by contact between the different placental cells.
2. The phenotypic mixing between HIV-1 and vesicular stomatitis virus (VSV) has been exploited to assay the susceptibility of human term syncytiotrophoblast cells to penetration by various strains of HIV-1. Results from our study suggest that syncytiotrophoblasts can be infected with cell-free HIV-1 and the susceptibility of these cells to penetration by the virus is strain dependent. The mechanism of entry of HIV-1 into syncytiotrophoblasts remains a challenging problem.
3. We demonstrated that interactions between ST cells and macrophages activated HIV-1 from latency and induced its replication in ST cells. In cocultures, ST cells delivered HIV-1 to the macrophages as evidenced by detection of virus-specific antigens in these cells. Direct contact between the different placental cells caused marked tumor necrosis factor-α and interleukin-6 release from macrophages. These cytokines induced productive replicative cycle of HIV-1 in ST cells.
4. Our findings indicate an interactive role for the ST layer and placental macrophages in the dissemination of HCMV and HIV-1 among placental tissue. Eventually, these interactions may contribute to the transmission of viruses from mother to the fetus.

This thesis is built on the following publications:

1. **Bácsi, A.**, Aranyosi, J., Beck, Z., Ebbesen, P., Andirkó, I., Szabó, J., Lampé, L., Kiss, J., Gergely, L., and D. Tóth, F.: Placental macrophage contact potentiates the complete replicative cycle of human cytomegalovirus in syncytiotrophoblast cells: role of interleukin-8 and transforming growth factor-β1.
   J. Interferon Cytokine Res. **19**, 1153-1160 (1999) IF: 2.171

2. **Bácsi, A.**, Ebbesen, P., Szabó, J., Beck, Z., Andirkó, I., Csoma, E., and D. Tóth, F.: Pseudotypes of vesicular stomatitis virus bearing envelope antigens of various HIV-1 strains permissively infect human syncytiotrophoblasts cultured in vitro.
   J. Med. Virol. **64**, 1-11 (2001) IF: 2.867

3. **Bácsi, A.**, Csoma, E., Beck, Z., Andirkó, I., Kónya, J., Gergely, L., and D. Tóth, F.: Induction of human immunodeficiency virus type 1 replication in latently infected syncytiotrophoblast cells by contact with placental macrophages: role of interleukin-6 and tumor necrosis factor-α.
   J. Interferon Cytokine Res. (in press) (2001) IF: 2.171

Other publications:

1. Szabó, J., **Bácsi, A.**, Andirkó, I., Kiss, J., Nemes, J., and D. Tóth, F.: Reciprocal interactions between human cytomegalovirus and human T cell leukemia-lymphoma virus type I in monocyte-derived macrophages cultured in vitro.
   AIDS Res. Hum. Retroviruses **14**, 699-709 (1998) IF: 2.499

2. Beck, Z., **Bácsi, A.**, Kovács, E., Kiss, J., Kiss, A., Balogh, E., Telek, B., D. Tóth, F., Andirkó, I., Oláh, É., Udvardy, M., and Rák, K.: Changes in oncogene expression implicated in evolution of chronic granulocytic leukemia from its chronic phase to acceleration.
   Leukemia and Lymphoma **30**, 293-306 (1998) IF: 1.140

3. Szabó, J., **Bácsi, A.**, Beck, Z., Kiss, J., Andirkó, I., and D. Tóth, F.: Role of interleukin 8 and transforming growth factor β1 in enhancement of human cytomegalovirus replication by human T cell leukemia-lymphoma virus type I in macrophages coinfected with both viruses.
   J. Interferon Cytokine Res. **19**, 209-217 (1999) IF: 2.171

4. Szabó, J., Beck, Z., Csomán, É., Liu, X., Andirkó, I., Kiss, J., **Bácsi, A.**, Ebbesen, P., and D. Tóth, F.: Differential patterns of interaction between HIV type I and HTLV type I in monocyte-derived macrophages cultured in vitro: Implications for in vivo coinfection with HIV type I and HTLV type I.
   AIDS Res. Hum. Retroviruses **15**, 1653-1666 (1999) IF: 2.499

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1. **Bácsi, A.**, Aranyosi, J., Szabó, J., Beck, Z., Ebbesen, P., D. Tóth, F.: Placental macrophage contact potentiates the complete replicative cycle of human cytomegalovirus in syncytiotrophoblast cells.
   International School for Molecular Biology and Microbiology (ISMBM), 1999, Smolenice, Slovakia

2. **Bácsi, A.**, Aranyosi, J., Ebbesen, P., D. Tóth, F.: Placental macrophage contact potentiates the complete replicative cycle of human cytomegalovirus in syncytiotrophoblast cells.
   3rd Annual Meeting of the European Society for Clinical Virology, 1999, Budapest, Hungary

3. **Bácsi, A.**, Aranyosi, J., Beck, Z., Szabó, J., D. Tóth, F.: Syncytiotrophoblast cells are permissive to the complete replicative cycle of human cytomegalovirus by contact with placental macrophage.
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4. **Bácsi, A.**, Aranyosi, J., Szabó, J., Beck, Z., D. Tóth, F.: The possible role of placental macrophages in the transplacental transmission of human cytomegalovirus. (in Hungarian)
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6. Szabó, J., Beck, Z., **Bácsi, A.**, D. Tóth, F.: Studies on the susceptibility of natural killer cells to HIV-1 strains of various tropism.
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7. Beck, Z., Szabó, J., **Bácsi, A.**, D. Tóth, F.: Induction of full replication cycle of human cytomegalovirus by Tax protein in $CD4^+$ cells.
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