HCMV Induces CCR5 Expression, Activation and HIV-infection at Feto-Maternal Interface

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Abstract

Background: Despite effective administration of combination antiretroviral therapy (cART) to HIV-1–infected pregnant women, mother-to-child transmission (MTCT) of HIV-1 remains a significant global public health concern. Co-pathogens that pose threats to the developing fetus include human cytomegalovirus (HCMV), may also facilitate *in utero* transmission of HIV-1 in populations where co-infection is common. HCMV infects >90% and 50%–80% of reproductive-age women in developing and developed countries, respectively. We hypothesize that HCMV promotes *in utero* transmission through increased immune activation, local inflammation, and maternal stimulation of fetal/placental CD4+ CCR5+ placental macrophages (or Hofbauer cells [HCs]) to increase cellular susceptibility to HIV-1. Strong associations between *in utero* HCMV infection and a higher incidence of MTCT of HIV-1 have recently been documented; however, the mechanisms that increase MTCT remain unknown.

Methods: With written informed consent, placentae and cord blood were collected from 15 HIV-1 and Hep. B seronegative women (>18 years) at Emory Midtown Hospital in Atlanta, GA. Peripheral blood was obtained from healthy adult volunteer donors. In this study, myeloid subsets were exposed to HCMV in vitro, followed by HIV-1BaL. Cells were also treated with IFN-α or IFN-β. Viral replication was determined by HIV-1 p24 ELISA. qPCR, FACS, and Western blot analysis determined the expression of CCR5, cellular activation markers, and antiviral ISGs. Cytokines and IFNs were measured by ELISA. Data were analyzed by using Student's t-test and Mann-Whitney test.

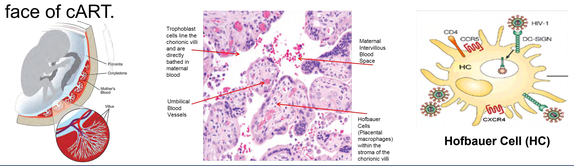
Results: HCMV exposure upregulated expression of the HIV-1 co-receptor CCR5, along with activation markers (CD16, CD80 and HLA-DR) on HCs. HCMV significantly induced the secretion of proinflammatory cytokines (IL-6 and TNF-α) by HCs, which enhanced their susceptibility to HIV-1 infection. We also showed type-I IFNs alone potently restrict HIV-1 replication in HCs, however HCMV infection inhibits Stat2 activation, which may override type-I IFN-induced protection to promote HIV-1 infection.

Conclusions: These data suggest that activation and local inflammation induced by maternal HCMV *in utero* alter CCR5 expression in macrophages at the feto-maternal interface to promote MTCT of HIV-1. We also present evidence that HCMV inhibits type-I IFN-dependent Stat2 signaling in the placenta, which may disrupt the intrinsic antiviral response in this compartment. Identifying biological determinants of MTCT provides a sentinel foundation upon which to launch rationally designed strategies for prophylaxis and the development of effective immunotherapies.

Background

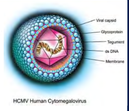
Mother-to-Child Transmission of HIV-1

- Annually, an estimated **199,000** new HIV-1 infections are expected to occur in exposed infants
- Even with strict adherence, maternal combination antiretroviral therapy (cART) reduces but does not eliminate transmission
- Most infants born to and nursed by HIV-1-infected mothers remain uninfected, however the risk of *in utero* transmission is only 7%
- However, maternal co-infections can overcome this barrier, even in the face of cART.



Background

Human Cytomegalovirus (HCMV)



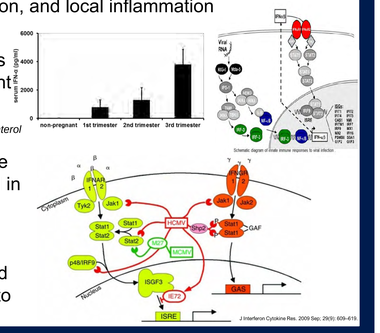
- A ubiquitous beta herpes virus, infects >90% of reproductive age women in developing countries
- Strong associations between *in utero* HCMV infection and a higher incidence of MTCT of HIV-1 have been noted AND congenital and postnatal CMV acquisition are strong independent correlates of vertical transmission of HIV-1

How Can HCMV Co-Infection Facilitate *in utero* Transmission of HIV-1?

- HIV-1 and HCMV can infect HCs and fetal myeloid cells, along with fibroblasts, epithelial, trophoblast, and endothelial cells.
- HCMV infection of the decidua promotes IFN-γ and IP-10 production, along with chronic villitis and trophoblast damage, providing the possible placental access by HIV-1 to target cells.
- HCMV reactivation is common in pregnant HIV-1-infected women and may promote HIV-1 transmission to the fetus through increases in proviral load, immune activation, and local inflammation

Type-I IFNs

- High Type-I IFN concentrations have been detected in pregnant compared to non-pregnant women *(Paternoster DM, et.al., J Clin Gastroenterol 2008)*
- High levels of type-I IFNs in the placenta have been implicated in fetal protection from herpes simplex virus and hepatitis C virus infection
- Type I IFN treatment in myeloid cells potently induces a block to HIV-1 infection

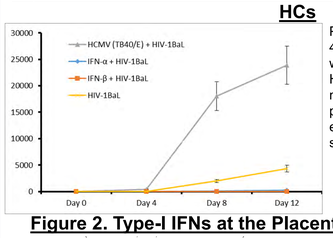


Hypothesis

We postulate the HCMV-induced activation and inflammation at the maternal-fetal interface promote MTCT of HIV-1

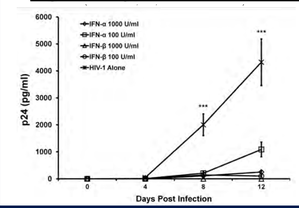
Results

Figure 1. HCMV/HIV-1 Co-Infection Promotes HIV-1 Replication in HCs



Placental HCs were infected with HCMV for 48h or treated with IFN-α or IFN-β for 24h (or with media alone), and then challenged with HIV-1_{BaL} over time. HIV-1 replication was measured in the cell supernatants by HIV-1 p24 viral antigen ELISA. Data shown are expressed as the mean ±SE of triplicate samples from 6 donors.

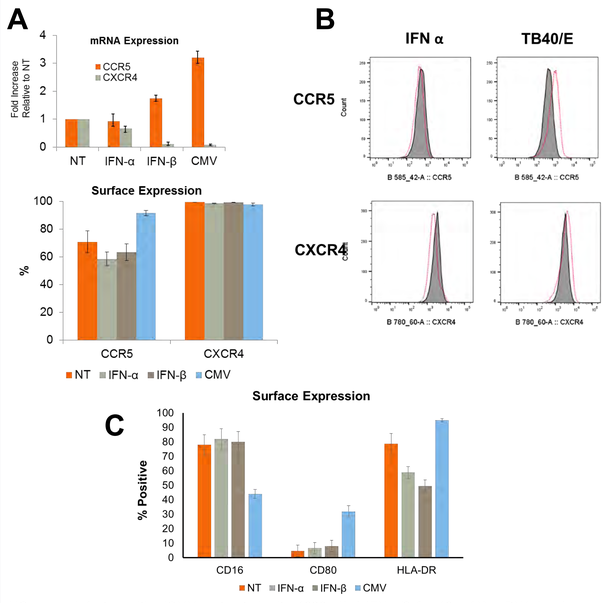
Figure 2. Type-I IFNs at the Placenta Limit HIV-1 Replication



Placental HCs were treated with IFN-α or IFN-β for 24 hours (or with media alone), and then challenged with HIV-1BaL over time. HIV-1 replication was measured in the cell supernatants by HIV-1 p24 viral antigen ELISA. Data shown are expressed as the mean ±SE of triplicate samples from 10 donors. Asterisks (*** p<0.001) indicate time points where values are significantly higher in untreated compared to IFN-treated HIV-1 infected HCs.

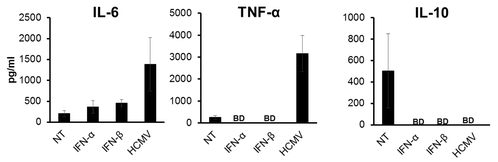
Results

Figure 3. HCMV infection Upregulate Surface Expression of CCR5, HLA-DR and CD80 on HCs.



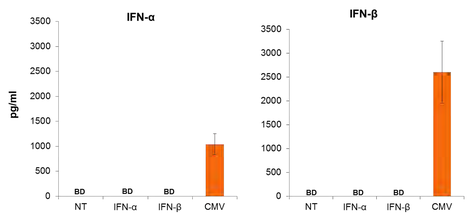
HCs were infected with HCMV or treated with 1000 U/ml IFN-α/β for 48h. (A) mRNA levels were measured after 24h by real-time PCR to determine the expression of CCR5 and CXCR4 relative to untreated controls. Data shown are expressed as the mean + SD of 7 donors. (B and C) CCR5, CXCR4, CD16, CD80 and HLA-DR surface protein expression was determine by flow cytometry.

Figure 4. IL-6 and TNF-α Secretion is Upregulated by HCMV



HCs were either infected with HCMV or treated with 1000 U/ml IFN-α/β for 48h. Supernatants were collected and measured for production of IL-6, TNF-α, and IL-10. Cytokines were measured using Quantikine ELISAs. Data shown are expressed as the mean ± SE of triplicate samples and are representative of 3 independent experiments from different donors.

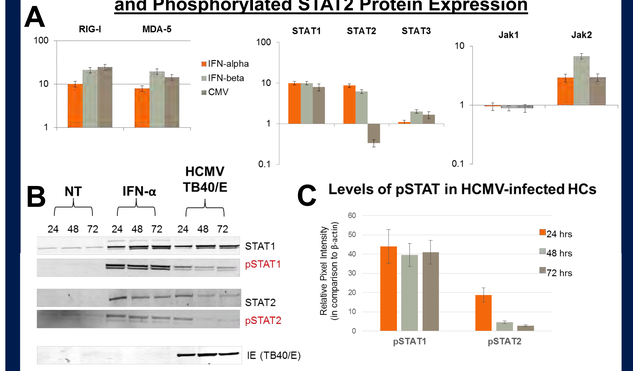
Figure 5. HCMV induces the secretion of type-I IFNs



HCs were either infected with HCMV or treated with 1000 U/ml IFN-α/β for 48h. Supernatants were collected and measured for production of IFN-α and IFN-β. Cytokines were measured using PBL ELISAs. Data shown are expressed as the mean ± SE of triplicate samples and are representative of 3 independent experiments from different donors.

Results

Figure 6. HCMV Infection of HCs Downregulates STAT2 mRNA and Phosphorylated STAT2 Protein Expression



HCs were infected with HCMV or treated with 1000 U/ml IFN-α/β for 48h. (A) The type-I IFN response was determined by measuring the mRNA levels of type-I IFN signaling proteins by quantitative PCR. (B) Endogenous and phosphorylated STAT1 and STAT2 protein expression was measured by quantitative western blot analysis. Data shown represent 7 donors.

Conclusions

- HCMV-induced CCR5 expression and activation may correlate with HIV-1-susceptibility in placental macrophages
- HCMV inhibits type-I IFN-dependent Stat2 signaling in the placenta, which may disrupt the intrinsic antiviral response in this compartment
- **Overall, these experiments will form a strong scientific basis for treatment of co-infection in the mother-infant dyad, which can significantly impact populations where HCMV and HIV-1 infection are highly endemic, optimizing current strategies for reducing MTCT of HIV-1**

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