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Human cytomegalovirus induces a distinct innate immune response in the maternal–fetal interface

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ABSTRACT

The initial interplay between human cytomegalovirus (HCMV) and innate tissue response in the human maternal–fetal interface, though crucial for determining the outcome of congenital HCMV infection, has remained unknown. We studied the innate response to HCMV within the milieu of the human decidua, the maternal aspect of the maternal–fetal interface, maintained *ex vivo* as an integral tissue. HCMV infection triggered a rapid and robust decidual-tissue innate immune response predominated by interferon (IFN)γ and IP-10 induction, dysregulating the decidual cytokine/chemokine environment in a distinctive fashion. The decidual-tissue response was already elicited during viral-tissue contact, and was not affected by neutralizing HCMV antibodies. Of note, IFNγ induction, reflecting immune-cell activation, was distinctive to the maternal decidua, and was not observed in concomitantly-infected placental (fetal) villi. Our studies in a clinically-relevant surrogate human model, provide a novel insight into the first-line decidual tissue response which could affect the outcome of congenital infection.

Introduction

Human cytomegalovirus (HCMV) is the leading cause of congenital infection, affecting 0.5–2% of newborns. Congenital HCMV disease develops in ~25% of infected children, and is associated with a wide spectrum of neurodevelopmental disabilities, intrauterine growth retardation (IUGR), and placental insufficiency (Cannon, 2009; Ross and Boppana, 2005). Our current understanding of HCMV transmission and pathogenesis is limited by the absence of animal models for congenital HCMV infection.

HCMV is transmitted from the mother to the fetus via the placenta (Pereira et al., 2005), which, in addition to serving in viral transmission, is actively injured by HCMV, contributing to the observed IUGR and fetal disease (Pereira et al., 2005, 2014; Schleiss, 2006, 2007). The initial steps of infection occur in the maternal decidua—representing the maternal aspect of the chimeric maternal–fetal interface, where virus originating from the mother amplifies before further spread to the adjacent placenta (Pereira and Maidji, 2008). The decidua is cohabited by the uterine microvasculature, invasive interstitial cytotrophoblasts, epithelial and stromal cells, as well as immune cells, with predominance of decidual NK cells (dNK) (Trundley et al., 2006).

Both immune and non-immune cells are known to be rapidly activated upon HCMV infection, with induction of innate immune response signaling cascades and inflammatory cytokines upon viral sensing (Mocarski et al., 2007; Rossini et al., 2012). Thus far, HCMV-induced innate responses have been studied mainly in single-cell-type cultures, such as fibroblasts and myeloid-lineage cells (Chaudhuri et al., 2009; Compton et al., 2003; Juckem et al., 2008; Mezger et al., 2009; Varani et al., 2007; Yurochko and Huang, 1999). In the context of congenital HCMV infection, adaptive immunity has clearly been more extensively evaluated than innate immune responses (Schleiss, 2013). While the potential contribution of innate responses to antiviral defense as well as to adverse tissue damage is increasingly recognized for diverse viral infections (Iwasaki and Medzhitov, 2013; Kobasa et al., 2007; Monroe et al., 2014; Rossini et al., 2012), their role in congenital HCMV transmission and outcome has remained elusive, in the absence of clinically-relevant model systems.

We have recently established a novel *ex vivo* model of HCMV infection in human decidual tissues maintained as multi-cell-type

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organ cultures, and demonstrated the spread and the broad target-cell range of the virus in the decidua (Weisblum et al., 2011). These findings reflected the ability of the *ex vivo* infected decidual culture to address the complexity of HCMV interactions with its diverse cellular targets within the natural tissue milieu. In the present work, we have employed the decidual culture model to study the decidual-tissue innate response to HCMV infection. Using both laboratory-adapted and clinically-pathogenic strains from congenital HCMV cases, we have identified a distinctive decidual tissue innate immune response pattern to HCMV infection.

Results

HCMV induces a robust decidual-tissue innate immune response

The decidual tissue is composed of multiple cell types in addition to the extracellular milieu. Its innate response to HCMV, as an integral tissue, is therefore likely to recapitulate the *in vivo* responses more accurately than individual cell types grown in culture. To evaluate the effect of HCMV infection on decidual cytokines/chemokines profile, we first analyzed the levels of representative immune and angiogenic cytokines/chemokines in cleared supernatants of HCMV-infected and mock-infected decidual organ cultures at 7 days post infection (dpi), using an ELISA immune assay (Table 1). To account for the tissue-to-tissue variability, mean values were calculated for 5 independent tissues obtained from different individuals. While the majority of the analyzed cytokines were not altered by more than ~2-fold following infection, HCMV substantially induced decidual secretion of interferon (IFN)γ (~35 fold; $p=0.03$), the T-cell attracting chemokine Interferon inducible Protein-10 (IP-10; 4.7 fold; $p=0.008$) and IFNβ (5.5 fold, not statistically significant). Similar induction of IFNγ and IP-10 were observed at 24 h post infection (hpi; data not shown).

In further comparative analyses, we quantified the effect of HCMV infection on decidual-tissue cytokines/chemokines mRNA levels, using quantitative real-time RT-PCR. In accordance with the secretion pattern, HCMV infection significantly induced decidual transcription of IFNγ and IP-10 as well as the interferon stimulated gene MxA (Fig. 1A) in all the decidual tissues examined (> 100), despite the expected tissue-to-tissue variations. Induction was similarly observed in tissues obtained from HCMV seropositive (64% of the total) and HCMV seronegative (36%) donors. No significant alterations of IL-10 and VEGF mRNA levels were observed (Fig. 1A).

To evaluate whether HCMV infection affects the tissue distribution of IP-10, histological sections of infected and mock-infected decidua were examined at 8 dpi (Fig. 1B). Immunohistochemical analysis revealed that in addition to the upregulation of IP-10 expression, HCMV infection drastically altered the distribution of IP-10 from distinct focal expression (in mock-infected tissues) to widely-distributed expression (in infected decidual tissues), detected in both infected and uninfected cells throughout the decidua.

Decidual tissue innate response kinetics

We quantified the HCMV immediate early (IE)-1 gene and the tissue cytokine mRNA levels over time. MxA, IFNγ, and IP-10 expression was already induced at 6 hpi, with continued increase reflecting the viral spread (Fig. 2).

Together, these findings reveal the rapid immune activation with creation of a proinflammatory environment in the decidual-tissue upon HCMV infection.

Induction of decidual innate response by viral particles

When decidual tissues were infected with 10-fold escalating doses of purified viral particles, a dose-proportional increase in IFNγ and IP-10 induction was demonstrated (Fig. 3A). The rapid and dose-dependent innate response of the decidual-tissue suggested that it was triggered by the viral particles immediately upon infection. We have therefore compared the induction of IFNγ and IP-10 expression following exposure to infectious *versus* UV-inactivated virions. Whereas no viral gene expression was detected following decidual exposure to UV-inactivated virions (Fig. 3B), a similar induction of IFNγ and IP-10 expression was observed, compared to infectious virions (Fig. 3B). This finding indicated that the induced decidual response does not require viral gene expression, but is rather triggered by virion structural component/s.

Furthermore, a similar innate response pattern was observed after infection with HCMV laboratory-derived strain AD169, the clinically-derived strain TB40/E, and the low-passage clinical isolates CI851, CI893, and CI943 (Fig. 3C), suggesting the potential triggering by conserved viral structural protein/s.

HCMV glycoprotein B (gB) is a major viral-envelope glycoprotein, implicated in the induction of cellular innate responses (Compton et al., 2003; Rossini et al., 2012). Incubation of decidual cultures with purified soluble gB resulted in a significant induction of IFNγ and IP-10 expression (Fig. 3D). This significant induction was similarly observed for each of 2 independent lots of mammalian cell-expressed gB (results are shown for one lot) and an unrelated preparation of baculovirus-insect-cell expressed and purified soluble gB, whereas no induction was observed following parallel incubation of decidual cultures with control glycosylated bovine serum albumin (BSA; Fig. 3D). These results identify the contribution of HCMV gB to activation of the decidual-tissue innate response.

Decidual-tissue innate response is already induced upon virus-cell contact

Neutralizing HCMV hyperimmune globulins (HIG) do not inhibit the decidual-tissue innate response

Clinical studies have suggested that treatment with HCMV HIG can reduce maternal–fetal transmission and congenital disease, although more recent controlled studies have not confirmed the effect on transmission (Nigro et al., 2005; Revello et al., 2014). We have shown that HCMV HIG efficiently neutralize HCMV infection of decidual organ culture (Weisblum et al., 2011). Here we sought to examine whether blocking the first steps of the virus replication cycle by neutralizing antibodies could also inhibit the viral-induced decidual innate response. Interestingly, despite the complete inhibition of infection (as revealed by the abrogated viral IE-1 expression), preincubation of purified viral particles with HCMV HIG had no effect on the decidual innate response to HCMV (Fig. 4A). As HCMV neutralizing antibodies have been shown to block the viral fusion and internalization step, but do not interfere with the initial virus binding at the cell surface (Compton, 2004; Potzsch et al., 2011)—our finding indicates that the decidual-tissue innate response is already activated upon the earliest stages of infection involving viral glycoprotein binding to cell surface receptor/s.

Heparin partially abolishes the decidual-tissue innate response. Initial viral attachment involves binding of viral envelope glycoproteins to cell surface heparan sulfate proteoglycans (HSPG), a process known to be inhibited by heparin. To investigate the contribution of virion—HSPG binding to the decidual-tissue innate response, purified HCMV was incubated with heparin prior to decidual infection. Heparin completely inhibited HCMV decidual infection, which resulted in a significant ($p<0.05$) albeit partial abolishment of IP-10 and IFNγ induction (Fig. 4B). No non-specific cytokine effects of heparin on the examined parameters were observed (Fig. 4B). This differential effect, identifies the relative contribution of viral recognition by cell surface