Human cytomegalovirus (HCMV) is the most common cause of congenital viral infection. Affected children can have permanent neurological complications, including hearing loss, visual impairment and mental retardation. In Australia, 57% of women are seronegative and at risk for primary infection and transmission of virus to the fetus during pregnancy. Despite its public health significance, the specific molecular and cellular basis of HCMV replication in the human placenta and pathogenesis associated with poor clinical outcome are unknown. Direct fetal infection is involved in severe cases of neuropathology and infection of the placenta can impair its development and functions resulting in a hypoxic environment and stillbirth. Gestational age at the time of infection is an important determinant of outcome. The rates of virus transmission increase from 30% in first trimester to over 70% in third trimester suggesting different mechanisms for overcoming the placental barrier. Remarkable insights into viral pathogenesis factors that function in the tissue environment have been gained by studying congenitally infected placentas and explants infected by clinical strains ex vivo. Together these studies revealed that direct infection of specialised placental cells and paracrine factors contribute to impaired development and functional defects.

As shown in Figure 1, the human placenta is composed of chorionic villi bathed in maternal blood and villi that anchor the placenta in the uterine wall (decidua), attaching the fetus to the mother (panel a1). The individual chorionic villus contains a connective core with blood vessels that carry substances to the fetal circulation. In floating villi, they fuse to form a multinucleate syncytiotrophoblast (STB) covering attached at one end to the tree-like fetal portion of the placenta. The rest of the villus floats in a stream of maternal blood, which optimises exchange of substances between the maternal and fetal circulation. In the pathway that gives rise to anchoring villi, cytotrophoblasts aggregate into cell columns of non-polarised mononuclear cells that attach to and then invade the uterine wall (ICTBs).
Our studies of HCMV infection in the human placenta revealed important differences in infection between cell types and maternal immune status. Studies of primary human placental cells and tissue models have identified molecular pathways that impair trophoblast differentiation. Patterns of viral proteins in infected placentas suggest modulation of infection in early...
gestation and formation of structural defects during pregnancy.

Congenital HCMV infection can result in intrauterine growth restriction (IUGR), which is found in conjunction with changes in the placental architecture. Specific pathology includes fibrinoids that occlude the villous surface, avascular villi and arrested differentiation of trophoblasts. Together these changes contribute to impaired transport functions, even without virus transmission to the fetus. A hypoxic environment evolves that up-regulates the vascular endothelial growth factor, its receptor and a soluble form, which is elevated in amniotic fluid and cord blood of infected babies.

We have utilised placental villus explants as a model to investigate the early steps in HCMV infection and found tissue effects not anticipated by studies in primary cells and have begun to identify viral pathogenesis factors for the human placenta. Specifically, we discovered that a clinical strain (VR1814) undermines the formation of cell columns in anchoring villi, but an attenuated laboratory strain (AD169) lacking a segment of the viral genome does not. These divergent abilities to replicate in cytotrophoblasts in villus explants were not observed in isolated cells infected with these viruses. In the placenta model system, the uninfected controls developed robust cell columns and anchoring villi of cytotrophoblasts that aggregated and attached the explants to the substrate. Surprisingly, explants infected with the attenuated strain formed normal-size anchoring villi indistinguishable from controls.

In contrast, explants infected with the clinical strain formed spindly cell columns composed largely of individual cytotrophoblasts that migrated on top of instead of invading the substrate. Analysis of cytotrophoblasts within the placental villi revealed that the attenuated strain infected few cells as indicated by low expression of the viral immediate-early (IE) IE1&2 proteins and failed to make gB, a late viral protein that signifies productive infection (not shown). In contrast, many cytotrophoblasts infected with the clinical strain expressed IE1&2 proteins and HLA-G was down-regulated, suggesting infected cells could become targets of natural killer cells in the decidua.

Since the attenuated and clinical strains exhibited markedly distinct levels of infection in placental explants, the differences were quantified by counting the number of cytotrophoblasts expressing IE1 protein in the cell columns and anchoring villi. AD169-infected explants contained a median of 2% infected cytotrophoblasts with a 5% maximum. In contrast, VR1814-infected placental villi contained a median of 26% infected cells with a 67% maximum. To quantify the effects on development of anchoring villi, we measured the sizes of villi formed by measuring the areas covered by the villous outgrowths (Figure 1m). Control explants and those infected with AD169 were comparable whereas explants infected with VR1814 formed significantly smaller villi less than 10% the size of controls. Together, the results showed that a clinical strain expressed pathogenesis factors that promote infection of cell column cytotrophoblasts and impair functions of cells that form anchoring villi, reducing their size in explants.

Important insights into virus replication in the tissue environment were also obtained using xenografts of human placental villi implanted under the kidney capsules of Scid-hu mice. Our immunohistochemical analysis revealed differences in the ability of pathogenic and attenuated HCMV strains to impair cytotrophoblast invasion, blood vessel remodelling and the development of a lymphatic vasculature. Moreover, cytokines important for lymphangiogenesis dysregulated by the clinical strain but not by the attenuated strain have functional effects in villus xenografts. These findings emphasise the critical importance of examining infection in the intact human tissues in order to understand viral effects on the developing placenta.

Future studies of viral replication in the natural tissue environment of the human placenta could provide insights into HCMV pathogenesis factors including tropism genes that modulate viral entry and enable the spread of infection impairing placental development.

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References


Under the Microscope
174 MICROBIOLOGY AUSTRALIA • NOVEMBER 2015

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