Cytomegalovirus (CMV) infection during pregnancy can have devastating effects on the developing fetus. Maternal CMV infection can affect the fetus in two ways: firstly by transmission to, and replication in, fetal tissue resulting in direct damage to developing organs; or, less well recognised, through cellular changes that potentially affect placentation and transfer of nutrients and gases to the developing fetus.

Congenital CMV infections occur in approximately 0.5-2% of births and 10-15% of these infections will be symptomatic, resulting in petechiae, jaundice, hepatosplenomegaly, chorioretinitis or more severe manifestations such as cytomegalic inclusion disease (CID) and stillbirth. As such, CMV has become the leading viral cause of congenital malformation in newborns now that rubella vaccination is universally available. More than half of CMV-infected infants who are symptomatic at birth are also at risk of long-term sequelae such as learning difficulties or sensorineural hearing loss. Symptomatic congenital infections are usually the result of CMV transmission in the first trimester of pregnancy. Fortunately, a larger percentage of children infected with CMV during pregnancy will remain asymptomatic. Less well recognised is the percentage of miscarriage and preterm births that may result from CMV infections in pregnancy. The reasons for these differences in outcome are unknown, but CMV strain variation, co-infections, host immunity and altered host cellular responses are suspected of playing a role.

**Congenital CMV infection and maternal immunity**

CMV infection of the placenta and fetus most often results from primary infection of the mother. CMV transmission is therefore facilitated by an immunologically naive host, allowing the virus to replicate to high titres in the infected mother, and disseminate and cross the placenta before a sufficient immune response is mounted. Low avidity antibodies with poor neutralising activity are generated following primary CMV infection and the presence of these antibodies in the first 20 weeks of pregnancy is a strong predictor for congenital transmission of CMV. Conversely, high avidity antibodies are produced much later through the process of immune maturation but provide greater neutralising ability and protection against CMV transmission. This explains why hyperimmune globulin therapy is able to reduce the incidence of congenital transmission when given to women with primary CMV infections.

A mature cellular immune response is also important in limiting CMV dissemination and controlling reactivation from latency. Despite this, secondary CMV infections in the mother have also been shown to contribute to a small but significant percentage of congenital transmission, suggesting CMV-specific maternal immunity is not always protective, particularly when a different strain of the virus is acquired. These factors have implications for the design of potential vaccines that must protect against infections with different CMV strains and provide a sustained humoral and cellular immune response during pregnancy. A DNA vaccine containing plasmids encoding the CMV pp65 phosphoprotein (a primary target of the host CD4+ and CD8+ T-cell response) and envelope glycoprotein gB (which elicits a strong T-cell response and neutralising antibodies) has recently shown promise in phase 1 clinical trials in CMV seronegative adults. However, like many candidate vaccines before it, enhanced CMV immunity in CMV seropositive individuals could not be achieved. 

**Transplacental transmission of CMV**

The human placenta is the primary route for transmission of CMV from mother to fetus. Maternal viraemia can result in spread of virus to the placenta, which serves as a reservoir for CMV replication and subsequent transmission to the fetus. The placenta offers some protection against congenital transmission,
consistent with our observations of CMV detection in placenta tissue without concomitant infection of the corresponding fetus or newborn. For CMV congenital infection to occur, the virus must negotiate a complex pathway across the placenta that involves a number of different cell types. The exact process of CMV transplacental transmission is not fully understood, but recent studies are providing valuable insights into the potential mechanisms and transmission routes of virus from mother to fetus.

Transmission of congenital CMV is dependent upon passage through specialised cells of the placenta called cytotrophoblasts. These cells have specific functions depending on their location within the placenta. Villous cytotrophoblasts located at the surface of floating chorionic villi act as progenitor cells for the formation of an outer multinucleate syncytiotrophoblast layer. This syncytiotrophoblast layer is in direct contact with maternal blood in the intervillous space and normally acts as a conduit for exchange of nutrients and gases for the developing fetus. Extravillous cytotrophoblasts arrange into columns of anchoring chorionic villi that attach to the uterine wall. Cells at the base of these columns further differentiate to become invasive cytotrophoblasts that enter the interstitium of the uterus and the uterine vasculature, diverting and increasing maternal blood flow to the placenta. Potential transmission routes of CMV across the placenta therefore involve passage through syncytiotrophoblasts of the chorionic floating villi and/or infection of extravillous cytotrophoblasts of anchoring villi.

We typically observe CMV DNA within syncytiotrophoblasts of CMV-infected placentae (Figure 1), with CMV also detected within underlying cytotrophoblasts, stromal cells and endothelial cells lining fetal vessels within the floating chorionic villi. This suggests that CMV can enter the fetal circulation by transfer from maternal blood across the syncytiotrophoblast layer to infect underlying cytotrophoblasts, before transmission through the stromal layer to fetal vessels.

CMV productively infects primary syncytiotrophoblasts in culture, but evidence that active CMV replication occurs within syncytiotrophoblasts in utero is limited. Examination of biopsies taken from early trimester placenta suggests that CMV is transported across the syncytiotrophoblast layer by receptor-mediated transcytosis of virion-antibody immune complexes utilising the pathway normally used to transfer maternal IgG for passive immunity.

Transmission of infectious virus is dependent on the virion-immune complex containing low avidity, rather than high avidity, antibodies. It is hypothesised that virion-immune complexes consisting of high avidity antibodies are transported intact across the syncytiotrophoblast layer, but are endocytosed by macrophages within the chorionic floating villi or internalised by underlying cytotrophoblasts. Conversely, CMV virions in complexes with low avidity antibodies are thought to be released when they reach the cytotrophoblast layer, and therefore able to go on and infect other cells and fetal blood vessels within the chorionic villi. This hypothesis is consistent with the higher incidences of congenital CMV transmissions that occur in the presence of low avidity maternal antibodies.

Evidence also exists for the transmission of CMV across the placenta through infection of invasive extravillous cytotrophoblasts of the anchoring villi from the maternal decidua. This route also appears to be a complex and lengthy pathway for transplacental transmission of the virus, but may be relevant in the early stages of gestation where the placenta is undergoing rapid changes.

The effect of CMV infection on placental development

CMV is known to induce changes in the expression of cellular proteins of infected cells and subvert many cellular pathways to

Figure 1: CMV localisation in the human placenta. CMV was detected by in situ PCR (purple) in syncytiotrophoblast (ST) cells (purple) lining placental floating villi (FV) (a). In normal pregnancy, the syncytiotrophoblast layer allows for transfer of nutrients from maternal blood in the intervillous space (IVS) to fetal blood vessels (FB). Control in situ PCR carried out on the same tissue demonstrates the reaction is specific for CMV (b).
advance or promote viral replication. It is therefore conceivable that CMV infection of the differentiating and supporting cells of the placenta can have deleterious effects on placentation and fetal development. Indeed, there is increasing evidence that CMV infection can indirectly affect the developing fetus by altering the process of placentation and causing pathological changes to the placenta in the early stages of pregnancy.

Early cytotrophoblast invasion of the uterine decidua is critical to the establishment and maintenance of a functioning placenta, and a number of proteins are known to be important in this process, including cellular growth factors, integrin receptors and matrix metalloproteinases. KAI1, a metastasis suppressor protein, is expressed by decidual cells at the uterine-placental interface, and thought to promote invasion of the endometrium by extravillous cytotrophoblasts. We have demonstrated increased expression of KAI1 in the decidua of CMV-infected placenta, suggesting CMV may interfere with the communication between invading fetal cytotrophoblasts and cells of the maternal decidua that is thought to regulate placental development.

In addition to this, CMV infection of extravillous cytotrophoblast cells directly interferes with their differentiation towards invasiveness, metalloproteinase-9 secretion and epithelial growth factor expression. We are currently investigating the extravillous trophoblast infectivity of low-passage CMV strains isolated from maternal urine and congenitally infected infants to determine the differential effects of strain variation and identify the viral genes essential for replication in cytotrophoblast cells.

The role of inflammatory cytokines

The role of cytokines in congenital CMV infection is only now being elucidated. Some groups have shown even UV-inactivated CMV can elicit the release of inflammatory cytokines and apoptosis in syncytiotrophoblast cells, suggesting cytokine mediated damage of the syncytiotrophoblast layer as a potential mechanism for CMV infection of placental villi and transmission to the fetus. Likewise, changes in fetal cytokine production in response to CMV infection may also have a role in the pathogenesis of CMV-induced fetal damage.

We have observed changes in the level of certain pro- and anti-inflammatory cytokines, including interleukin-6, in the amniotic fluid of CMV-infected fetuses. These changes have correlated with cytokine expression in extravillous trophoblasts and epithelia of the amniotic membrane (Figure 3). Further experiments are underway to determine whether observed changes in fetally-derived cytokine levels are similar to changes in CMV-infected placental tissue at different stages of gestation, and to correlate these with histopathological tissue damage.

Animal models for the study of congenital CMV transmission

The investigations of human CMV congenital infection described above are helping to elucidate the mechanisms of transplacental CMV transmission but there are obvious ethical and practical limitations to in vivo studies of human CMV pathogenesis. Human CMV is highly host restricted and will only infect humans and human cell lines, and animal models are therefore required to study CMV infection in vivo.

There are many animal homologues of human CMV, but only a few of these have been extensively studied. Guinea pigs and rhesus macaques are similar to humans in terms of placental architecture and congenital CMV infection, but use of these animal models is limited in terms of availability, practicality and what we know about guinea pig and rhesus CMV in general. Much more detailed knowledge is available regarding the pathogenesis, immunology and genetics of murine CMV infection in mice, and murine models are used extensively to advance our understanding of human placental development and other factors affecting pregnancy outcomes. Transplacental transmission of murine CMV from immunocompetent mice to fetal pups has not been previously demonstrated, although congenital infection of fetal pups can occur when murine CMV is injected directly to murine placentas.
Developments in the small animal models of guinea pig and mice are likely to be seen in the near future. These models will complement ongoing investigations of human CMV and assist with identification of therapies and vaccines for prevention of CMV transplacental transmission and congenital infection.

References
22. Smith, L.M. et al. (2008) Laboratory strains of murine cytomegalovirus are genetically similar but phenotypically distinct from wild strains of virus. J. Virol. 82, 6089-6096.

Dr Gillian Scott completed her PhD on cytomegalovirus antiviral susceptibility and resistance in 2004 and is now a postdoctoral scientist in the Department of Microbiology, SEALs at Prince of Wales Hospital and conjoint lecturer at the University of New South Wales. She has continued her research of CMV susceptibility to current and potential antiviral agents as well as investigations of CMV pathogenesis in liver transplantation and congenital infection.

Alicia Steller, Shu Wang and Karen Teng are currently honours students at the University of New South Wales conducting research into CMV congenital infection at Prince of Wales Hospital. Alicia intends to pursue a career in science following completion of her studies, Shu will continue her medical studies at UNSW and Karen intends to continue as a PhD student next year.

Sharon Chow is a UNSW PhD student in the final stages of her studies into maternal and fetal immunological responses to congenital CMV infection. The majority of her research is conducted in the virology research lab at Prince of Wales Hospital under the supervision of Prof William Rawlinson in collaboration with Prof Cheryl Jones from the Children’s Hospital at Westmead.

Figure 3: IL-6 expression in amniotic membranes. IL-6 expression (brown) is localised to the extravillous trophoblasts (ET) and amniotic epithelium (AE) of fetal amniotic membranes (a). Immunohistochemistry negative control (no primary antibody) carried out on tissue from the same individual indicates IL-6 detection is specific (b).