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Cytomegalovirus, infections in pregnancy and congenital infection

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Cover image: Human cytomegalovirus (CMV) infection of the placenta. CMV-infected placental tissue stained for CMV immediate early IE1p72 protein (red), cellular vimentin (yellow) and nuclei stained with DAPI (blue). Image kindly provided by Dr Stuart T Hamilton.

Jonathan Iredell
President of ASM

Increasing diversity in the general area of Microbiology is welcome and inevitable. The modern microbiologist is a mathematician or an experimentalist, an ecologist or a cell biologist, a geneticist or a population biologist. Rather than concern ourselves with a narrowing definition, we must aim for inclusiveness and opportunity. Maree Overall in the National Office and Cheryl Power, our Vice President (Corporate affairs), have undertaken an analysis of the declared interests of the members of the Society (many Society members listing more than one interest, of course), and there are obvious groupings of several hundred each.

We have long used a Divisional structure to bring cohesion and improve representation of the special interests within the Society, and these have included the relatively cohesive Division Four (Molecular Microbiology) and Division Two (Virology) and the highly diverse Divisions Three and One. Division Three has included eight special interest groups and Division One has included nine. The breakdown of members' interests is shown in Table 1.

Some interest groups are naturally linked and align within existing Divisions but it may be that new areas need to be freed of traditional divisional links. As a common example, bioinformatics and genomics includes everything from traditional whole genome sequencing of pathogens of public health/agricultural importance through to metagenomics of large systems and what some might call 'systems biology', and is increasingly integrating both new analytical approaches and new data types well beyond representational/descriptive analyses of nucleic acid and protein sequences. Some with special interest in the microbiology of the gut microbiome, for example, might have more in common with colleagues who work in ocean systems than they do with traditional alignments inside Divisions One or even Four.

We once more call all teachers, scientists and researchers in the general field of Microbiology to consider what grouping are appropriate to better represent and serve your needs and to make representations to either your State Branch Chairs or Divisional Chair, or direct to the national office simply entitled 'ASM review submission'. All these will be considered over the next

Table 1.

Declared interests of combined membership	*n*	Current division
Antimicrobial	718	1
Clinical Microbiology; Serology; Diagnostics; Mycology; Mycobacteriology	886	1
Parasitology and Tropical Medicine; Veterinary Microbiology	636	1
Virology	436	2
Cosmetics and Pharmaceuticals, Culture Media; Food Microbiology	771	1/3
Public Health	606	1
Education; History	768	3
Ecology/Environmental		3
Informatics		3/4
Molecular Microbiology/Bacterial Pathogenesis	787	4
Bioinformatics/Genomics	?	1–4

several months and brought for discussion to national Council and to the Annual General Meetings. What new meetings do you need ASM to support? What issues do you want to discuss?

The American Society of Microbiology President has expressed sentiments I think we all share and I would urge MA readers to look at her essay in mBIO (http://mbio.asm.org/content/6/5/e01573-15) and at Vertical Transmissions in previous editions of this publication (March and September 2015).

One possibility to consider is that we look at (say) an increased number of divisions, perhaps including groupings such as those tabulated above. We welcome submissions to the national office (admin@theasm.com.au) and these will be considered in the larger review. Each division would look toward meetings and visiting speakers as the initial concrete expression of their common interests.

Congenital cytomegalovirus: the invisible problem

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It is a great pleasure mixed with some sadness to write this editorial. The entire November issue is around the subject of congenital infection, with the focus on the most common, serious cause of congenital malformation in Australia – congenital cytomegalovirus. Infection with cytomegalovirus (CMV) causes serious disease in children globally, resulting in congenital infections present in ~2000 Australian newborns every year, of whom most are asymptomatic, with ~450 per annum (pa) affected by hearing loss, mental disability and other illnesses. Some of the key clinical features of congenital infection are outlined here in articles by Wendy van Zuylen, Klaus Hamprecht and Robert George, and the pathogenetic features in Lenore Pereira's paper. Treatment and vaccination are moving ahead (as discussed in papers from some key Italian groups), although not fast enough for many of us – as parents of children with CMV discuss in two papers here. We also include papers on other causes of congenital infection that are much less common than congenital CMV in Australia. Although these are not related to congenital CMV clinically, with very different medical and epidemiological settings, it is important to put congenital CMV in context, as well as to draw attention to other important causes of congenital infection.

We all love children, and we all hate to see them distressed or unwell. When their illness arises during pregnancy, from an infection, and is avoidable, we are all troubled, and then can become disillusioned. Why if this is avoidable was this allowed to happen? Why did it happen to this (my) child? Why is there no treatment? Why is there little policy in this area? And why are we informed about seafood (potential mercury and infection risks), soft cheese (potential listeria risks), undercooked meat and pregnant cats or kittens (toxoplasmosis risks), sexually transmitted infections (HIV, syphilis) and not about CMV? It is important to put these public health messages in context, when the rates of these other infections are much lower. In Australia annually, for HIV there are ~1.3 new paediatric congenital infections pa, listeria there are ~15 new paediatric infections pa, syphilis there are ~7 new congenital infections pa, and for congenital toxoplasmosis the rates are unknown, but on clinical likelihood less than 20 per year. Direct quotes from families affected by congenital CMV are the best way of telling the story – 'I really wish that I wasn't writing to you and I wish that I wasn't aware about a virus called CMV. However this isn't the case', 'My doctor told me about CMV after our baby was born', 'When I asked about treatment and was told there was none available, and the only one that might work was experimental, I wondered why'.

So what is our responsibility as health care workers, researchers, parents, aunts, uncles, grandparents and policymakers? The authors here from scientific, medical, clinical obstetric, paediatric, infectious diseases, and family backgrounds address some of these issues. They outline not only the problems we see clinically, scientifically, and personally but also some things that can be done now to stop pregnant women from getting CMV and from their babies suffering from illnesses caused by congenital CMV infection. These include informing parents, obstetricians, midwives, allied health professionals and other clinicians about congenital CMV, increasing research into preventing CMV, producing vaccines, producing antivirals, and lobbying for better policy now in stopping CMV. Indeed there are things we can do now to prevent the tragedies of congenital CMV, again in the words of a parent whose child was stillborn as a result of congenital CMV – 'I still don't have all the answers as to what happened or why things were not known. This will not help E, but maybe it will help someone else'.

Finally, we have a tribute to Professor Geoffrey Shellam. Geoff was a friend and colleague to us, whose achievements were in many areas of virology and immunology, not only CMV. However, for those of us working in CMV infection, he represented a great strength, friend and mentor, particularly working with the murine model of CMV pathogenesis. Indeed '*A muribus discimus hominum – From mice man learns*', and Geoff along with his outstanding group of researchers at UWA learnt a lot from mice, but also transmitted that knowledge to many of us, including me. He is greatly missed, but leaves an honourable legacy of respect, knowledge and love that will continue.

All of the authors here hope that through the articles in this publication our understanding of congenital CMV can be shared, our enthusiasm for solving this terrible medical problem can encourage engagement of clinicians and researchers, and ultimately we can help prevent congenital CMV from affecting our children in the future.

Biography

Professor William Rawlinson is a Medical Virologist and is Director of the Division of Serology and Virology (SAViD) and a NSW State Reference Laboratory in HIV, in the Department of Microbiology SEALS. He is a consultant position to the Department of Infectious Diseases, Prince of Wales and Sydney Children's Hospital. He holds a conjoint academic position as Professor in the School of Medical Science and the School of Biotechnology and Biomolecular Sciences at The University of New South Wales, currently supervising PhD, Masters and Honours students. His major research interest is in human cytomegalovirus (CMV) infection of mothers and babies, particularly mechanisms of transplacental virus transmission. The research group that he heads studies congenital infections, enteroviruses, hepatitis viruses, respiratory viruses, novel antivirals and antiviral resistance.

Clinical and epidemiological features of congenital cytomegalovirus infection globally

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Human cytomegalovirus (CMV) is the most common non-genetic cause of congenital disability. As a herpesvirus that infects the majority of the population, CMV is able to establish a lifelong latent infection in the host. Any time during pregnancy, a primary CMV infection, reactivation of latent CMV or a new viral strain can infect the placenta and the developing foetus, resulting in congenital CMV infection. Each year, an estimated 2000 children are born with congenital CMV infection in Australia, leaving ~500 children with permanent disabilities such as hearing or vision loss, or mental disability. Despite the clinical importance of congenital CMV, there is limited awareness and knowledge in the medical and general community about congenital CMV infection. This article reviews the global epidemiology and clinical features of maternal and congenital CMV infections.

Human CMV infection

Human CMV is a member of the *Herpesviridae* family of viruses, which includes Herpes simplex virus type 1 and type 2, Varicella zoster virus, Epstein-Barr virus, Human herpesvirus 6A, Human herpesvirus 6B, Human herpesvirus 7, and Human herpesvirus 8[1,2]. The genome of human CMV is ~235 kbp and is one of the largest among the *Herpesviridae*[3].

Human CMV infects most individuals in the world and can be acquired anytime during life: as a foetus, neonate, toddler, child or an adult. Initial infection (also known as primary infection) occurs following close personal contact. CMV is typically transmitted via body fluids, particularly breast milk, urine, genital secretions, and blood[4]. In addition, CMV can infect the placenta and the developing foetus[5]. Once infected, the human body does not clear the virus. CMV is able to persist in a latent form in either low or undetectable levels in peripheral blood mononuclear cells ($CD14^+$) and bone marrow cells ($CD34^+$ and $CD33^+$)[6]. Stimuli such as inflammation, immune impairment due to pregnancy, medical treatment with immunomodulating agents such as corticosteroids, chemotherapy, and immunosuppressive therapy post organ transplantation may stimulate reactivation and growth of latent CMV[7]. Considering CMV secretion in urine and cervical-vaginal fluids increases during pregnancy with increasing gestational age, hormonal changes related to pregnancy may also stimulate reactivation of CMV[8].

Epidemiology of maternal and congenital CMV infections

CMV is a common cause of infections worldwide. Antibodies to CMV, representing a previous infection, can be detected in 45 to >90% of women of reproductive age[9]. The percentage of women that are infected with CMV varies between countries and tends to be the lowest in Western Europe, Australia, Canada and the United States and the highest in South America, Africa and Asia[9]. Particularly, in Australia, the average seroprevalence rate of CMV for women between the ages of 14 to 44 years is 58%[10]. However, even within countries the rate of CMV infected women varies by socio-economic status and ethnicity[9,11].

Approximately 1–2% of initially uninfected pregnant women will acquire CMV by the time of delivery[12]. A possible source of CMV for these women is young children whose saliva and urine contain high levels of CMV[13]. In addition, a partner who is infected with CMV is an additional possible risk factor for infection during pregnancy, as CMV is present in semen, and can be transmitted sexually[12]. Among the women who acquire a primary infection during pregnancy 32% transmit CMV to the foetus via the placenta, resulting in congenital CMV (cCMV) infection[14]. Only a percentage of cCMV infected children will exhibit symptoms at birth or develop CMV associated symptoms later in life, as further described in detail below.

The foetus can also be infected by a woman's latent virus or re-infection with a different strain of CMV (secondary infection)[15]. The risk of transmitting CMV to the foetus is reported to be higher when a pregnant woman acquires a primary infection during the first half of the pregnancy compared to secondary infections, or infection in

the second half of pregnancy[16]. Kenneson[14] reported 1.4% of secondary infections lead to foetal infection. However, considering the high seroprevalence of CMV, it is estimated that more than two-thirds of CMV infected children are born to mothers who were already infected with CMV[17].

Intrauterine CMV infection occurs in 0.2 to 2% (average of 0.64%) of live births in the Unites States, Australia and Western Europe (Table 1)[14,18–20]. In addition, the limited studies of regions in Latin America, Africa, and Asia have reported a birth prevalence of cCMV infection ranging from 0.6 to 6.1% of pregnancies[20]. Based on the number of live births per year[22] and reported cCMV prevalence[14,18–20], this translates to an estimated ~0.12 million cCMV infections in developed countries per year, and ~0.7 million to 4.5 million cCMV infections annually in developing countries. Particularly in Australia, an estimated ~2000 children are born with cCMV infection in Australia each year (Table 1). Nonetheless, in practice, most congenital CMV infections remain undiagnosed[18].

Clinical features of maternal CMV infection

The majority of CMV infections in immunocompetent individuals do not cause symptoms; however, clinical manifestations could include glandular fever (mononucleosis) syndrome characterised by flu-like symptoms, or occasionally persistent fever[24]. Several studies reported that pregnant women, who acquired a primary CMV infection, experienced mononucleosis, fever, fatigue, and headache[25]. Additionally, Nigro[24] observed a significantly higher number of pregnant women with primary CMV infection presenting with symptoms compared to pregnant women with recurrent or latent CMV infection. A review of congenital CMV cases in Australia reported more than half of the mothers had evidence of, or could recall experiencing symptoms of fever during pregnancy[26]. In addition to clinical symptoms, laboratory examination may show an increase in lymphocytes in the blood and increased serum levels of liver enzymes (alanine transaminase and aspartate transaminase)[24]. Since all of these clinical manifestations are not only observed upon a CMV infection, they do not represent specific indicators of maternal CMV infection. However, collection of the clinical history and laboratory examination may be extremely useful for dating the onset of infection to determine the risk of CMV transmission to the foetus and risk of cCMV disease.

Clinical features of congenital cytomegalovirus disease

A minority (~10%) of cCMV infected children present symptoms at birth (Table 2). Physical signs such as petechiae, jaundice, and hepatosplenomegaly are common and have been observed in 28 to 50% of children with cCMV infection[18,27]. Neurological abnormality, including microcephaly and intracranial calcification has been reported to occur in 18–38% of cCMV infected children. The majority of these affected children develop sensorineural hearing loss, mental disability, motor deficits, chorioretinitis and seizures[16,23,26].

A significant amount (~15%) of initially asymptomatic CMV infected children will encounter developmental difficulties, neurological problems, or hearing loss before the age of five[9,18,28]. Among those with hearing loss ~40% of children may develop severe to profound

Table 1. Estimated annual number of cases of cCMV infection in Australia, United States, Western Europe, Latin America, Africa, and Asia.

Region	Live births per year (in 1000s) (LB)	Estimated rates of CMV infection per 100 live births (R)[A]	Estimated number of live births with cCMV infection (*N* = R*LB)	Symptomatic cCMV infection at birth (S = *N**10%[A])	Asymptomatic cCMV infection at birth – later symptoms (AS = *N**15%[A])	Estimated total number of live births with cCMV associated symptoms (*t* = S+AS)
Australia	~308[B]	0.64%	1971	197	296	493
United States	~4334[C]	0.64%	27 737	2774	4160	6934
Western Europe	~1900[C]	0.64%	12 160	1216	1824	3040
Latin America	~11 746[C]	1.9%	223 174	22 317	33 476	55 794
Africa	~41 024[C]	3.4%	1 394 816	139 482	209 222	348 704
Asia	~79 738[C]	3.95%	3 149 651	314 965	472 448	787 413

[A]Average percentage rates based on data from Kenneson and Cannon[14], McMullan *et al.*[18], Munro *et al.*[19] and Lanzieri *et al.*[20].
[B]2013 data from the Australian Bureau of Statistics[21].
[C]Data from *World Population Prospects: The 2012 Revision*[22].

Table 2. Clinical symptoms of congenital cytomegalovirus infection[18,23,26].

• Petechiae and jaundice
• Hepatosplenomegaly
• Microcephaly and intracranial calcification
• Chorioretinitis and vision loss
• Sensorineural hearing loss (SNHL)
• Mental disability, motor disabilities, and seizures

impairment of both ears[23]. Other neurological complications such as microcephaly, neuromuscular defects, and chorioretinitis may also develop in initially asymptomatic CMV infected children, but at a lower rate compared to symptomatic infection[1].

Congenital CMV infection may also result in adverse pregnancy outcomes, as cCMV has been associated with fetal death *in utero*, neonatal death, preterm birth and maternal pregnancy complications, including preeclampsia[29–33].

Concluding remarks

CMV continues to be the leading infectious cause of congenital malformation in developed countries. More children may be affected by cCMV than by any other childhood disorder, such as down syndrome, fetal alcohol syndrome, and spina bifida. Each year in Australia, an estimated 2000 children are born with cCMV infection, leaving ~500 children with permanent disabilities such as hearing or vision loss, or mental disability. Even though the rates of maternal and cCMV infection are still lacking for many parts of the world, which likely underestimates the global impact of cCMV infection, the importance of cCMV infection and disease as a large public health problem is self-evident.

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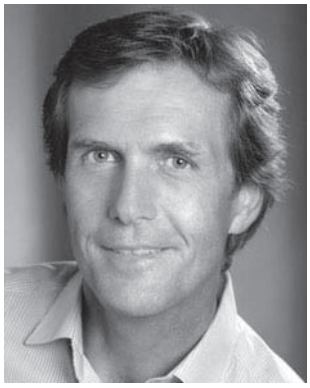
Biography

Wendy van Zuylen is a postdoctoral scientist in the School of Medical Sciences, Faculty of Medicine at the University of New South Wales. Her current research at the Virology Research Laboratory aims to understand the pathogenesis of congenital cytomegalovirus infection.

Therapeutics to prevent congenital cytomegalovirus during pregnancy: what is available now and in the future?





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Human cytomegalovirus (CMV) is the leading non-genetic cause of fetal malformation in developed countries. Congenital CMV infection can cause serious clinical sequelae, and in severe cases result in fetal or neonatal death. Despite the clinical and social importance of congenital CMV there is currently no standardised management strategy to prevent or treat maternal/fetal CMV infection during pregnancy and no evidence-based therapeutic for prenatally diagnosed CMV infection or disease. For pregnant women with a primary CMV infection during pregnancy, standard medical practise remains to offer no treatment at all or the option to terminate pregnancy. If intervention is requested, pregnant women may be offered a narrow range of medical therapies with limited evidence for efficacy and some with high risks of toxicity. However, there are several experimental and novel anti-CMV therapeutics currently being investigated that may provide a safe and effective therapeutic for use during pregnancy to prevent both fetal infection and reduce the risk of congenital CMV disease developing in the fetus once infected *in utero*.

Established anti-CMV therapeutics

Valaciclovir

To date, the only established CMV antiviral to undergo investigations during pregnancy is valaciclovir, the prodrug of the nucleoside

analogue aciclovir. The converted active form of acyclovir is incorporated into the replicating viral DNA causing premature chain termination. Valaciclovir is used as a CMV prophylaxis in organ transplant recipients[1]. A pilot observational study showed maternal treatment with oral valaciclovir to treat confirmed cases of fetal CMV infection was well tolerated and decreased viral load in fetal blood[2]. Furthermore, treatment resulted in a modest decrease in adverse fetal outcomes (52% of 21 infants) compared with the untreated group (58% of 24 infants). These results led to a French multicentre, nonrandomised, single group assignment, phase IV clinical trial evaluating the efficacy of valaciclovir in the treatment of confirmed fetal CMV infection, which has recently completed[3]. Results demonstrated a high safety profile with no liver or renal toxicity observed and met the primary endpoint in reducing the number of symptomatic children at birth and number of terminations of pregnancy for fetal anomalies using the Optimal Two-Stage Simon' design. The outcome of valaciclovir treatment was 34/43 (79%) asymptomatic neonates, 2/41 (5%) terminations of pregnancy and 7/43 (16%) symptomatic neonates. Due to the toxicity associated with the other established CMV antivirals (ganciclovir, valganciclovir, cidofovir and foscarnet) it is unlikely these will ever be investigated in any clinical trials involving pregnant women.

Hyperimmune globulin (HIG)

Immunoglobulin therapy has been used safely during pregnancy for passive immunisation against a wide variety of viruses including; cytomegalovirus, rubella, varicella, measles and hepatitis A and B. To date, there have been several clinical trials, observational studies and case series reports on the prophylactic and therapeutic use of HIG during pregnancy, which has been recently reviewed in detail[4]. While all the various studies on using HIG to prevent or treat congenital CMV to date have shown a beneficial trend, the lack of data from properly designed randomised clinical trials limits the conclusions that can be drawn and prevents recommendation as a

Table 1. Current antiviral therapeutics for cytomegalovirus infection.

Drug	Mechanism of action	Administration/ (TGA class[A])	Indication
Valaciclovir	Inc. into the viral DNA causing premature chain termination	Oral (Category B3)	CMV prophylaxis in transplant recipients, experimental use in CMV-infected pregnant women
Ganciclovir/ Valganciclovir	Inc. into the viral DNA causing premature chain termination	Intravenous/Oral (Category D)	CMV treatment, pre-emptive therapy, or universal prophylaxis, too toxic for use during pregnancy
Foscarnet	Inhibits viral DNA polymerase by blocking pyrophosphate binding site	Intravenous (Category B3)	Second-line agent for treatment of infection due to ganciclovir-resistant CMV, too toxic for use during pregnancy
Fomivirisen[B]	Anti-sense RNA which acts as a translational inhibitor of CMV immediate early mRNA	Intraocular (N/A)	Second-line agent for local treatment of CMV retinitis, unsuitable for use during pregnancy
CMV HIG	Pooled CMV IgG antibody, from high titre donors which transfers passive immunity	Intravenous (Unlisted)	CMV prophylaxis in in organ recipients or for CMV severe disease. Experimental use in CMV-infected pregnant women
Cyclopropavir	Inc. into the viral DNA causing premature chain termination	Oral (Unlisted)	Investigational at this stage
Cidofovir/ Brincidofovir	Inc. into the viral DNA causing premature chain termination	Intravenous/Oral (Category D)	Cidofovir used as second-line agent for treatment of ganciclovir-resistant CMV; Brincidofovir remains investigational
Maribavir	Inhibits viral nuclear egress and efficiency of virus production by binding to the CMV kinase pUL97	Oral (Unlisted)	Investigational at this stage
Letermovir	Targets CMV terminase complex and inhibits cleavage and packaging of CMV progeny DNA into capsids	Oral (Unlisted)	Investigational at this stage
Artesunate	Signalling inhibitor that particularly interferes with cellular activation pathways (NF-κB and others)	Oral (Unlisted)	Treatment for pregnant women with severe malaria, investigational at this stage for CMV infection

[A]Toxicity and tetragenicity risk to fetus from the Australian Government, Department of Health, Therapeutic Goods Administration[5].
[B]Recently withdrawn from the market due to infrequent medical use and lack of economic viability.

standard therapeutic for congenital CMV during pregnancy. Of all the established and experimental CMV antiviral therapies currently available, only HIG and valaciclovir have been investigated in pregnant women (Table 1).

Novel therapies with clinical drug candidates

Brincidofovir

Brincidofovir (or CMX001) is an alkoxyalkyl ester analogue of cidofovir (a nucleoside analogue of cytosine), which acts by competitive inhibition of cysteine incorporation into the viral DNA strand and thus causing premature chain termination. While no data are available for the safety and efficacy of brincidofovir to prevent or treat congenital CMV, several studies in animal guinea-pig models show cidofovir and brincidofovir have potential benefits in preventing CMV transmission during pregnancy[6–8]. A recent phase II study on the efficacy of brincidofovir to prevent CMV disease in haematopoietic-cell transplant (HSCT) recipients showed promising results[9]. Patients who received brincidofovir twice weekly had significantly fewer incidence of CMV events than those who received placebo (10% *v.* 37%; $P = 0.002$). Brincidofovir has now entered phase III trials in HSCT recipients.

Maribavir

Maribavir is an orally bioavailable benzimidazole antiviral that binds to the CMV-encoded protein kinase pUL97 (a viral orthologue of cellular cyclin-dependent kinases) and inhibits viral nuclear egress and the efficiency of virus production in CMV-infected cells. Despite early phase I and II clinical trials showing promising anti-CMV activity observed[10,11], this activity could not be confirmed in two subsequent phase III prophylaxis trials performed in allogeneic HSCT[12] and liver transplant recipients[13]. However, there is evidence that supports increasing the dosage of maribavir may improve efficacy[14]. Shire are currently conducting two Phase II dose-ranging clinical trials in transplant recipients for first line treatment of CMV viremia and treatment of resistant/refractory CMV. Resistance against maribavir has been reported at times, but the clinical significance has still to be investigated in the ongoing Phase II studies. Interestingly, the residues subject to resistance mutation within the pUL97 kinase are distinct from those of ganciclovir/valganciclovir resistance, although all contained within the kinase domain (Figure 1). Notably, other pUL97 inhibitors, presently under intense investigation at the experimental level, do not show detectable resistance formation[15,16].

Letermovir

Letermovir (AIC246) represents a new class of non-nucleoside CMV inhibitors known as the 3,4-dihydro-quinazolines. Letermovir acts by targeting the CMV terminase complex and thus interfering with viral DNA concatemer maturation and subsequent cleavage and packaging of CMV progeny DNA into capsids. As there is no mammalian counterpart of the viral heterodimeric terminase enzyme, target-related toxicities, which are observed with the current anti-CMV polymerase inhibitors, are not expected[17]. Furthermore, the novel mode of action should provide new treatment options for resistant variants; however, the putative frequency of viral drug resistance to letermovir has not been addressed in detail[18]. Letermovir exhibits potent anti-CMV activity *in vitro* and *in vivo* and retains high efficacy against resistant variants. Letermovir has shown promising anti-CMV activity in an open-label, proof-of-concept phase IIa trial involving kidney/pancreas transplant recipients with

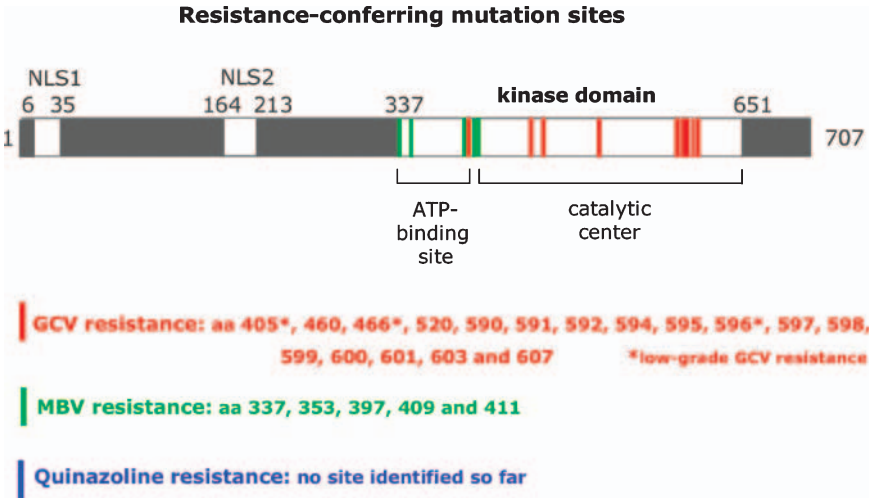

Figure 1. HCMV-encoded protein kinase pUL97 as a novel target of antiviral therapy: profile of mutations conferring drug resistance. Clinical and experimental treatment of HCMV infections led to the definition of hot spots of drug resistance formation, all localised within the pUL97 kinase domain. Ganciclovir/valganciclovir resistance mutations are displayed as red bars (hot spots at catalytic centre); maribavir resistance mutations as green bars (hot spots at ATP-binding site); no resistance mutations against experimental pUL97 inhibitors of the quinazoline class have been identified so far.

CMV viremia[17]. It also met all primary endpoints as a prophylactic drug in a recent phase IIb clinical trial in HSCT recipients and is currently entering phase III trials.

Artesunate

Another novel approach focuses on CMV inhibitors derived from natural resources or semi-synthetic derivatives like the antimalaria drug artesunate. Artesunate is derived from arteminisin, a natural product from the Chinese herb *Artemisia annua*. Meta-analyses of malaria patients treated with artemisinins demonstrated the safety of this class of drugs[19–21]. For pregnant women with severe malaria, artemisinins are recommended by the World Health Organization as first-line therapy during the second and third trimester whereas less certainty about the safety is given in the first trimester[22]. In addition to antimalaria activities, artesunate possesses a strong and broad antiviral activity and is particularly efficacious against CMV[23–27]. Based on promising data obtained *in vitro* and from an experimental animal model[28], a small number of clinical antiviral investigations in transplant recipients have been conducted so far. In some cases, treatment of patients with standard drug-resistant CMV led to an efficient control of infection[29,30], whereas other case reports showed an unsatisfying outcome with poor benefit or complete treatment failure[31,32]. A recent investigation using an *ex vivo* model of first trimester placental CMV infection showed a reduction of infection by 40% in the presence of artesunate[33]. Although the mode of action is not fully elucidated, the targeting of artesunate to cellular proteins was demonstrated[34–36] and may act as an inhibitor of cellular activation pathways, in particular the NF-κB pathway (C. Hutterer and M. Marschall, unpublished data).

Experimental therapies presently under early development

Kinase inhibitors

Small molecules inhibiting cyclin-dependent kinases (CDKs) or other virus-supportive cellular kinases show promise as a potential tool for host cell-directed antiviral intervention. CDKs are serine/threonine protein kinases characterised by the formation of heterodimeric complexes with cyclins thereby regulating substrate recognition and phosphorylation activity. An obvious advantage of the targeting of CDKs or other cellular kinases over direct antivirals (e.g. viral kinase inhibitor maribavir) may be the reduction, or even complete suppression, of the induction of drug-resistant virus as it is generally difficult to select for resistance to inhibitors that target cellular functions. This may improve the therapeutic quality of a novel drug candidate. The recent identification of a potent selective CDK7 inhibitor with broad anti-herpesviral activity and low cytotoxicity profiles nicely substantiates this concept[37] and now awaits proof-of-concept investigations in animal models. Thus, the kinase inhibitor strategy may lead to a novel option for antiviral therapy approaches that already proved to be highly potent in current anticancer therapy.

Inhibitors of viral nuclear egress

In vivo, HCMV production is largely co-regulated by the interaction between viral and cellular proteins and by the formation of virus-host multi-protein complexes. Recently, the viral 'nuclear egress complex' (NEC) has attracted the deep interest of researchers, since it represents a regulatory key position of viral replication and a

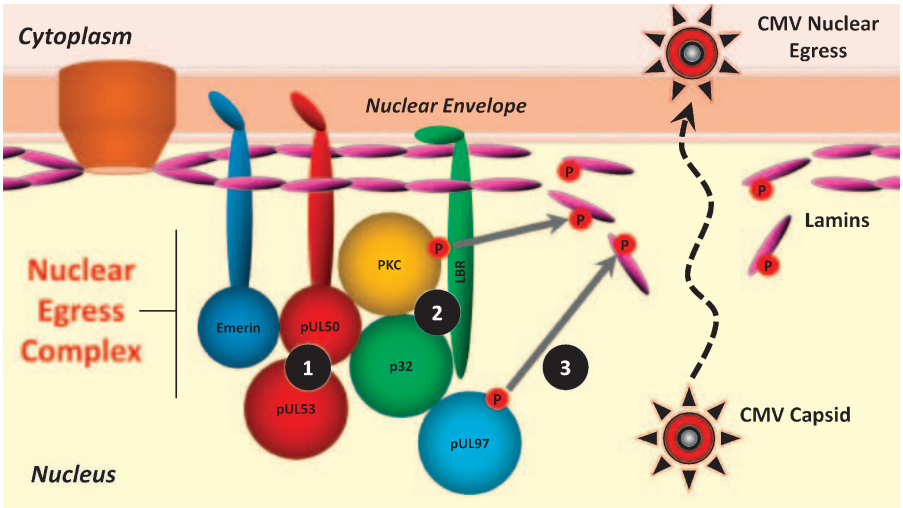

Figure 2. The postulated cytomegalovirus nuclear egress complex (NEC) and potential novel therapeutic targets. Recruitment of viral and cellular components leads to phosphorylation (P) and partial disassembly of the nuclear lamina, allowing viral capsids to be packaged and exported to the cytoplasm through the nuclear envelope. Potential therapeutic targets include: (1) blocking formation and/or expression of the core viral NEC components pUL50 and pUL53; (2) preventing subsequent recruitment of viral/cellular components of the NEC; and (3) inhibiting NEC phosphorylation and subsequent disassembly of the nuclear lamina.

putative target for novel antiviral strategies[16,38–41]. During the nuclear phase of HCMV replication, viral capsids are packaged and exported to the cytoplasm by transition through the nuclear envelope (nuclear egress) for further virion maturation. Nuclear egress is a multi-step regulatory process that involves a phosphorylation-triggered distortion of the nuclear lamina[42–45]. Pivotal for nuclear egress is the role of the two viral nuclear egress proteins pUL50 and pUL53 that heterodimerise and form a core for the multimeric viral-cellular NEC[46] (Figure 2). For antiviral strategies directed to the NEC as a novel target, two concepts are favoured at present, either (1) the development of small molecules (*in silico* drug docking analyses combined with high throughput screenings) that sterically interfere with NEC subunit interactions or (2) the identification of kinase inhibitors that potentially inhibit the phosphorylation of NEC components in order to block their ordered core or multimeric association in an indirect manner. First experimental candidates of NEC-inhibiting drugs have already been initially described by several researchers so that an increase of experimental and clinical data in this field is expected for the near future.

Conclusion

In the absence of a CMV vaccine or an evidence-based therapeutic option available to clinicians to prevent or treat fetal CMV infection during pregnancy, further scientific data is urgently needed on the safety and efficacy profiles of experimental and novel antiviral compounds. These investigations are extremely problematic given potential drug toxicities and the fact that CMV tropism and placental physiology are both highly host-specific. We have therefore begun investigating these experimental compounds in our established *ex vivo* placental explant model systems[47] to better inform future directions in clinical trials.

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Biographies

Dr Stuart Hamilton is a postdoctoral scientist in the Virology Research Laboratory, SEALS Microbiology at the Prince of Wales Hospital and University of New South Wales. His research is focussed on: (1) understanding transmission modes of intrauterine CMV infection; (2) examining the adverse effects CMV infection has on placental development/function and subsequent pregnancy outcomes; and (3) investigating safety and efficacy profiles of experimental CMV antiviral compounds in human *ex vivo* placental explant histoculture and cell culture models.

Dr Corina Hutterer is a senior postdoctoral scientist at the Institute for Clinical and Molecular Virology, Friedrich-Alexander University of Erlangen-Nürnberg, with a strong interest in drug development and novel intervention strategies directed against herpesviruses. She completed her PhD in virology at the Institute of Medical Virology at the Humboldt University of Berlin in 2010 and then joined the research laboratory of Manfred Marschall focusing on virus-host interactions and the development of better therapeutic options for the treatment of cytomegalovirus infections.

Professor Manfred Marschall is group leader and international expert in herpesviruses at the Institute for Clinical and Molecular Virology, Friedrich-Alexander University of Erlangen-Nürnberg. His major research interest is human cytomegalovirus and is particularly focussed on three major topics: (1) the functional and structural characterisation of the CMV-encoded protein kinase pUL97 and CMV nuclear egress complex; (2) the cross-talk between herpesviral and cellular kinases (i.e. the regulation of viral nuclear egress, the virus-CDK interregulation, as well as questions of virus-induced cellular signalling and pathogenesis); and (3) the development of kinase inhibitors (and others) as putative broad-spectrum antiviral therapeutics in collaboration with international biotech and pharmaceutical companies.

Reducing congenital cytomegalovirus infection through policy and legislation in the United States

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Policy and legislation, backed by accurate science, are viable tools to change behaviour to reduce congenital cytomegalovirus (CMV) infections. Addressing CMV through public policy can provide increased awareness among public health officials, access to existing venues for disseminating information, and much needed funds for awareness campaigns. While some medical professionals and CMV experts oppose public policy and legislation mandating medical practice, most support policies aimed at public education campaigns to provide consumers with accurate CMV education.

Changing behaviour through public policy

A woman's risk of becoming infected with CMV and transmitting CMV to her unborn child can be reduced when she practices hygienic precautions[1–4]. However, in the United States, only 13% of women are aware of CMV and only 44% of OB/GYNs counsel women about CMV and prevention measures[5,6]. This article explores the feasibility of increasing CMV awareness and prevention through public policy measures.

There are several policy strategies to promote healthy behaviours[7]. Strategies include:

- Providing information about the desired behaviour (point-of-decision prompts, mass media campaigns).
- Offering incentives/disincentives for behaviour (tax deductions, vouchers).
- Requiring/prohibiting behaviour (vaccinations, screenings).

The behavioural change theory that underlies most public policy is the rational choice model. People assess the choices before them in terms of costs and benefits and then select the choice that maximises their net benefits[8]. Incentives and disincentives can be very effective. Taxes on plastic bags have been extremely successful, leading to a 90% reduction in the consumption of plastic bags in Ireland[9]. Reports from the World Bank show that increasing taxes on tobacco sales is the single most important step governments can take in reducing smoking[10].

Governments also provide information to citizens to modify behaviour using the underlying assumption of the rational choice model: if people know that a behaviour and/or activity has adverse consequences they will reduce its incidence or eliminate it. Examples include tackling drinking and driving, HIV, drugs, child safety and smoking[8].

Public policy also addresses public health issues through required actions. These include required and recommended screening panels conducted for each newborn, regulated by countries, hospitals and clinics, and by each State in the United States.

One public health issue successfully addressed through public policy and legislation is the timely identification of childhood hearing loss. In 1988, the average age in the United States for the identification of hearing loss in children was 2.5 years.[11] As a result of the introduction of the newborn hearing screening, the average age of diagnosis was reduced to 3.9 months.[12]

One of the major contributors to such a dramatic shift in newborn care practice was state-based legislation (Table 1). In 1993, 3% of United States infants were tested for hearing loss at birth. By 2001, 80% were screened[13].

Utah's CMV public health initiative

In March 2013, the State of Utah passed a 'Cytomegalovirus Public Education and Testing' law requiring a CMV public health initiative. This law[14] requires:

(1) The Utah Department of Health to establish and conduct a public education program to inform pregnant women and women who may become pregnant regarding the incidence of CMV; the transmission of CMV to pregnant women and women who may become pregnant; birth defects caused by congenital CMV; methods of diagnosing congenital CMV; and available preventative measures.
(2) The Department of Health to provide the information to: child care programs; school nurses; school health education

Table 1. Evolution of newborn hearing screening in the United States.

1970	Not recommended, no viable test
1989	Rhode Island Demonstration Project screened 1850 babies
1990	First State law requiring Newborn Hearing Screening in Hawaii
1992	Second State law in Mississippi
1993	Three additional State laws passed; 3% of infants screened
1993 and 1996	Federal grants funded by the US Government to assist hospitals and states to implement Newborn Hearing Screening
1998	712 US Hospitals conducting Newborn Hearing Screening
1999	National Early Hearing Detection and Intervention (EHDI) program established by the US Congress
2001	All 50 States have EHDI programs; 80% of infants screened
2006	Hearing included on the Recommended Universal Screening Panel

Data obtained from http://infanthearing.org/legislation/

providers; health care providers offering care to pregnant women and infants; and religious, ecclesiastical, or denominational organisations offering children's programs as a part of worship services.

(3) If a newborn infant fails the newborn hearing screening test(s) a medical practitioner shall:

(a) test the newborn infant for CMV before the newborn is 21 days of age, unless a parent of the newborn infant objects; and

(b) provide to the parents of the newborn infant information regarding birth defects caused by congenital CMV and available methods of treatment.

Utah's law accomplishes two main objectives that will lead to reduction of CMV infections in mothers and infants. First, it establishes the Utah Department of Health as an authority on CMV and requires the Department to make information available to the public and professionals. The law makes it more likely that women in Utah will receive accurate information about CMV and how to prevent it. The law also contained a fiscal note, dedicating US$30 000 each year to the CMV public education program.

Utah's law requires CMV testing of infants who fail the newborn hearing screening. By requiring an action on behalf of the parents and the medical provider, the initiative creates additional awareness of CMV, which will lead to CMV prevention as well as appropriate and timely interventions (medical and therapeutical including speech therapy, occupational therapy and physical therapy.

Utah's CMV public health initiative has provided for advertisements in and on public transportation, in publications, and on social media to reach pregnant women and women who might become pregnant. Examples of their outreach can be found on their website, http://health.utah.gov/cmv, and their Facebook page: https://www.facebook.com/CMVUtah.

Other CMV legislation in the United States

Following Utah's successful legislation, five additional states have pursued legislation. Four passed legislation in 2015.

- Connecticut passed legislation in 2015 that does not include a public education program, but requires CMV testing for all infants failing the newborn hearing screening[15].
- Tennessee's legislature did not pass proposed legislation that mirrored the Utah law. Department of Health and medical association officials testified against the legislation[16].
- Hawaii passed legislation in 2015 requiring a public education program[17].
- Illinois passed legislation in 2015 requiring a public education program and CMV testing for infants who fail the newborn hearing screening[18].
- Texas passed legislation in 2015 requiring a public education program[19].

Parents and professionals have expressed interest in pursuing legislation in additional states in 2016 (personal communication, January to June 2015). It is not unrealistic to expect CMV legislation to be implemented in each of the United States within the next five to eight years.

One key to the successful CMV legislation in Utah was the partnership between policymakers, CMV experts and medical professionals, and advocates including parents and other family members impacted by CMV[20]. Without the input and advice of similar partners in other states including CMV experts and medical professionals, I anticipate it will be difficult to both pass and implement legislation.

Global CMV policy survey

In 2015, 30 medical professionals with experience studying or treating CMV experts from 24 countries participated in an online survey to assess consensus on statements related to support for potential CMV public health policy. Participants were recruited from participant lists from international CMV conferences and through recommendations from other professionals (S. Doutre and J. Greenlee, unpublished data).

Most CMV experts surveyed support government (74%) or professional (90%) policy requiring pregnant women or women who may become pregnant be counselled about CMV. Experts report they would support government (58%) or professional (58%) initiatives requiring screening of newborns for CMV. If these experts serve as

quality sources of information to policymakers and public health implementation personnel, such policy will serve as an effective tool in increasing CMV education, awareness and prevention.

Conclusion

While not a singular solution to CMV prevention, public policy can be a tool to increase awareness and prevention by both disseminating accurate information and requiring action by way of CMV testing. Increased agency attention, including via funding, to CMV will increase awareness and education among pregnant women, which may lead to reduction of congenital CMV. In the United States, five states have enacted CMV legislation requiring public education programs, targeted CMV testing or both. I anticipate the number to continue to increase with the support of CMV experts.

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Biography

Sara Menlove Doutre is a PhD student in the Psychology Department at Utah State University in Logan, Utah, USA and a research assistant at the National Center on Hearing Assessment and Management. She is also an Education Policy Consultant that advises education and health agencies on policy issues related to children with disabilities. Her daughter, Daisy, is deaf due to a congenital CMV infection. She is a co-founder of the Utah CMV Council and was influential in the passing of CMV legislation in the State of Utah and hosting the 2014 CMV Public Health and Policy Conference.

Congenital cytomegalovirus and its consequences for families

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Congenital cytomegalovirus (cCMV) is the most common viral and infectious cause of disabilities to newborn babies. It can cause sensorineural hearing loss and deafness, cerebral palsy, verbal, oral and motor dyspraxia, global developmental delay, microcephaly, feeding issues requiring a gastrostomy tube, intellectual disabilities, epilepsy, blindness and death. There are also children with cCMV who are on the autism spectrum however studies have yet to be carried out in this area. For the rest of the family the consequences of cCMV are life changing. Additional pressure on families, separation and divorces between parents, financial hardship, health issues such as anxiety, depression and chronic back problems are just a few examples. Siblings can often find themselves in carer roles, they will often have less time with their parents and are also at risk of having their own health issues such as anxiety.

Cytomegalovirus (CMV) is called a stealth virus for good reason. So many people often carry the virus without ever feeling the effects of it. Despite this CMV can be devastating to anyone with a weakened immune system. The surreptitiousness of the virus is also evident in the fact that often parents won't be aware that their baby has had the virus until they are diagnosed with a disability after they are born. Ironically this is usually a time in our lives which we expect to be one of the most beautiful – welcoming a new life into the world. Instead families can feel as if their lives have been turned completely upside down and violently shaken. Shattering everything we thought we knew into thousands of pieces which we are then left to try and put back together as best we can.

Some families mourn a miscarriage, others a still birth, and then there are others who might get a day or two with their babies before they die. For many of the more fragile children and their families there are a lot of hospital stays. These families live with the constant and unrelenting fear of not knowing how long their children have. They are the bravest people I know.

For those of us who have not lost our babies we know how lucky we are but our norm becomes filled with appointments with all sorts of early intervention therapists and medical specialists including Audiologists, ENTs, Paediatricians, Neurologists, Psychologists, Ophthalmologists, Infectious disease specialists, Gastroenterologists, Occupational Therapists, Physiotherapists, Audio Verbal Therapists, Speech Therapists, Special needs educators, Counsellors and Social Workers. We become case co-ordinators and fierce advocates for our children, cochlear implant trackers and under the direction of our children's therapists we also get pretty good with different types of therapy and special education. This becomes our main job, although it is not a job any of us would choose.

The specialists we see for our children do not have a crystal ball and so usually there are not many answers that can be given but our questions and fears are relentless, particularly in the first few years. Will our baby have an intellectual disability? Will they have seizures? Can my child hear me? Will he be able to hold his head up? Does she have cerebral palsy? Will he ever sit and support himself? Will she be able to feed herself? Is his hearing deteriorating? Will she ever crawl? Will he ever walk? Will she be able to make friends? Will we ever get him out of nappies? Will she ever talk? Does he understand me? Will we ever be able to reason with her? Will the tantrums ever stop? How big is the gap going to be? What kind of life is my baby going to have?

My son, William, is deaf with mild cerebral palsy, motor, oral and verbal dyspraxia and moderate global developmental delay. He and

his twin sister, Emmaline, turn 5 in November this year. For the first few years of William's diagnosis I was often an emotional mess, feeling quite disconnected from friends and family who were getting on with their lives. I found anxiety, depression and weight gain more difficult to manage as life became more overwhelming than I could have ever imagined. I have felt so isolated and alone at times, often the only people I felt really understood were other Mums who have gone through something similar. I know that I am one of many who will always wish that we had been given the opportunity to reduce our risk of contracting CMV by adhering to a few behaviour modifications while pregnant. We missed a 50% chance of preventing our children from having congenital CMV. I look forward to one day celebrating the implementation of a successful vaccine. Until then however I believe it is everyone's responsibility to make sure that all woman are counselled about congenital CMV before pregnancy. As the Hippocratic Oath says: 'I will prevent disease when ever I can, for prevention is preferable to cure.' www.cmv.org.au

– Kate Daly

The real story of congenital cytomegalovirus

Imagine that you, or your partner, have given birth to a baby and you find out that your baby has been exposed to a dangerous virus during the pregnancy. This virus could have a questionable impact on your newborn's development and prognosis. This virus is cytomegalovirus (CMV) and your newborn has been diagnosed with congenital CMV.

You were never informed of this virus by your physician during pregnancy and were never told of any precautionary measures that could possibly prevent exposure. You may blame yourself for allowing harm to come to your unborn child and may wonder how and where you may have acquired the virus and what you could have done to prevent it.

As you inform friends and family members about your newborn's diagnosis, they may ask questions about how you contracted this virus and what may happen to your baby. When you express your concern and may not have answers to their questions, they may search on the internet and find information regarding herpes and HIV, which could be confusing and can be taken out of context.

Your family and friends may also read about shedding and contagion issues and assume that your child could infect healthy people and cause them to experience disability or death. You may see a decline in contact from your friends and family. Upon learning of your baby's diagnosis, family and friends who expressed eagerness to visit and meet your baby may cancel their visits, not call at all or never speak to you again.

You may be disinvited from playgroups out of fear for the other children. There may be an assumption that your child could infect and disable otherwise healthy children in the group. You may have to field questions from babysitters and daycares about your child's diagnosis and about the perceived impact that your child's presence may have on the caregiver, the other children, and on their business.

When your child begins preschool or elementary school, there may be concern expressed about your child's diagnosis. You may have difficulty in explaining to the teachers and staff that your child poses no threat to anyone in the classroom or on the campus.

Over the months and years, you may also experience grief, shame, anger, and depression. You may wonder why other parents and medical professionals didn't sound their voices louder to warn you to possibly spare you and your child.

Fortunately, you are only asked to imagine this as an experience in your life and in your child's life.

As a parent of twins born with congenital CMV, I can attest to you that the disease burden of congenital CMV is profound and very real. While emerging research is giving us great insights into the epidemiology, prevention, diagnosis, and treatment of congenital CMV, the true and lasting impact of this virus is felt day after day in families the world over. Whether children are born mildly or severely affected by CMV, they, as well as their families, deserve a public health outcry and development of sound professional and government policy to help stop CMV.

– Janelle Greenlee

Biographies

Kate Daly is the Founder and President of the Congenital CMV Association of Australia, the only not-for-profit organisation in Australia focussing on congenital CMV, representing and supporting families affected as well supporting those working towards a cure. Kate lives in Sydney with her husband and four children.

> *I started the association in the hope that other parents won't feel as alone as I did when William was diagnosed and also to raise awareness about CMV so that other parents can have an opportunity I didn't, to minimise their risk of contracting the virus and giving it to their unborn babies.*

Janelle Greenlee is the founding president of Stop CMV, the world's largest all-volunteer organisation dedicated to CMV. Comprised of families, friends, and medical professionals personally affected by CMV, the mission of Stop CMV is to prevent and eliminate congenital CMV and to improve the lives of all people affected by congenital CMV.

New diagnostics and methods of assessing pregnant women at risk of cytomegalovirus



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Human cytomegalovirus (CMV) infection can occur in pregnant women by primary infection or by non-primary infection, namely by either reactivation of the latent virus or reinfection with a different strain[1]. In all cases the mother can transmit the virus to the fetus through the placenta[2,3]. In the diagnosis of primary CMV infection, the gold standard is maternal seroconversion to CMV-specific antibodies. Currently, women are not routinely screened for CMV before conception or during pregnancy, thus CMV seroconversion is infrequently documented[1]. Lastly, serological diagnosis of non-primary CMV infection is very difficult and very often unreliable since no optimal diagnostic methods are currently available. Today, the fetal compartment can be only studied by amniocentesis and ultrasound examination for the diagnosis and prognosis of CMV infection and generally, invasive diagnostic protocol can be only suggested to pregnant women with evidence of primary CMV infection acquired early in gestation and in case of abnormal findings suggestive of congenital infection[1]. Therefore, a correct maternal diagnosis makes so that invasive prenatal diagnosis is only offered in selected cases. This report points out how a CMV-screening program combined with an advanced diagnostic protocol performed on pregnant women could identify those at high risk of transmitting the virus to their fetus. Furthermore, we evaluated the possible role of soluble HLA-G (sHLA-G) molecules detected in maternal and fetal samples in order to more accurately assess a greater risk of CMV-transmission and fetal/neonatal injury.

Diagnosis of maternal CMV infection

Testing for anti-CMV IgM antibodies is the most widely used and appropriate procedure for screening pregnant women[4]. Anti-CMV IgM antibodies are a good indicator of acute or recent infection, however it is not always correlated with active infection[4–6]. The rate of CMV-IgM detection by screening test ranged from 3–5.7%[7–9], however only 7.5% of IgM-positive women have a congenitally infected fetus/newborn[1]. Consequently, all pregnant women with a positive screening CMV-IgM test should be offered advanced diagnostic testing as early as possible in pregnancy (before week 12–16 of gestation). Anti-CMV IgG avidity testing[10–13], CMV-IgG and IgM immunoblotting (IB)[14–16], microneutralisation assay[17], and detection of viral DNA in blood, saliva and urine samples[18–20] with Real Time PCR are currently the most reliable advanced procedures in order to identify all pregnant women who can transmit CMV infection.

The value of advanced diagnostic tests for identifying women at high risk of transmitting the virus

Since 1994, pregnant women with test results showing seroconversion or IgM screening positivity were referred to our centre for further analysis[19]. In 2014, we performed a check up on 194 pregnant women during a 6 month period. Out of these 194 cases 104 (53.6%) did not know their CMV serostatus before pregnancy. At the time of recruitment, the patients were in the first or second trimester of pregnancy, except 28 who were in the third trimester (range 6–38 weeks gestation, median 14).

We tested blood, saliva and urine samples obtained from all 194 women and were able to identify five different groups using serological and virological advanced diagnosis. In the first group of 15 non-immune pregnant women, all samples were positive/borderline for CMV-IgG or IgM with the screening assays. After using the advanced tests, we obtained in all cases negative results with IB-CMV IgG and IgM antibodies, undetectable IgG-avidity and CMV-DNA negative in all body fluids.

In the second group of 68 patients with past infection, the screening tests identified borderline/positive results for IgM antibodies in all 68 cases. After using advanced testing, we obtained negative results in all cases for IB-IgM antibodies and CMV-DNA in all body fluids. Moreover, we found high avidity in all cases.

In the third group, advanced diagnosis was able to identify 57 pregnant women with primary CMV infection. Also in this group the screening test was IgM positive or borderline and the IB confirmed this specific IgM-positivity for CMV. IgG-avidity in all 57 samples was low/moderate and in 52 out of 57 patients (91.2%) viral DNA in whole blood, saliva and urine samples was detected.

In particular, we found CMV-DNA in 35 whole blood samples out of 52 (61.4%) and the number of copies ranged from <500 to 8700 copies/mL. Higher rates of positivity were detected in the saliva and urine samples, 75.4% and 64.9%, respectively. The range varied from <500 to a maximum of 44 000 copies/mL of saliva and <500 to 9700 copies/mL of urine. Positive viral detection and viral load in whole blood, saliva and urine were not associated with a greater risk of infection and/or fetal/neonatal injury.

The fourth group included 43 pregnant women with non-primary CMV infection. IB confirmed the positive results for IgM in 40 out of 43 cases; in the remaining three patients we were able to prove the diagnosis of non-primary CMV infection with the detection of viral DNA in urine and saliva. In 43 pregnant women with non-primary CMV infection, we observed a good sensitivity of virological tests in saliva and urine samples (48.8% and 41.9%, respectively) and very low sensitivity in whole blood samples (9.3%). When considering overall virological results, we found viral DNA in body fluids in 65% of patients with non-primary CMV infection. The DNA levels were very low in all body fluids, ranging from <500 to 900 copies/mL in urine and less than 500 copies/mL in both whole blood and saliva.

Finally in the last group, the advanced diagnosis investigation confirmed active CMV infection, however we were not able to identify the kind of infection, hence the reason why this group included 11 pregnant women with undefined CMV infections. The incidence of congenital CMV infection in the 5 groups of pregnant women classified with advanced diagnostic protocol is shown in Figure 1.

Although generally the diagnosis of CMV infection remains complex, major goals have been achieved in recent years including maternal diagnosis with serological and virological tests. In particular, the use of advanced serological diagnosis has proven to be reliable in assessing pregnant women at risk of CMV infection. Likewise,

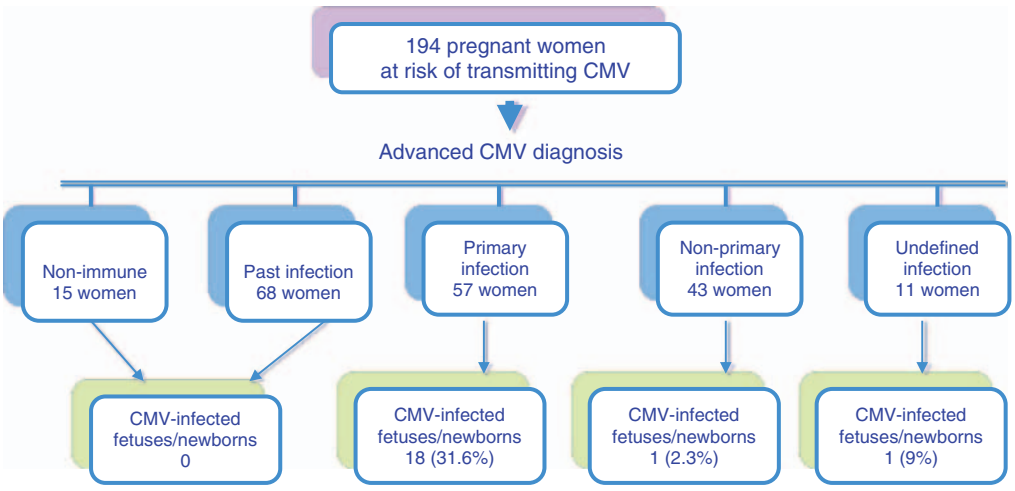

Figure 1. Prevalence of congenital CMV infection in 194 pregnant women at risk of transmitting the virus after serological screening.

virological diagnosis is also reliable and can support the serological diagnosis of primary, past and undefined CMV infection, as well as playing a role in the diagnosis of non-primary CMV infection.

Interaction between HLA-G expression and CMV infection during pregnancy

In order to improve the identification of i) pregnant women who transmit the virus to their fetus and ii) CMV-infected and compromised fetuses, we studied the expression of soluble isoform of HLA-G (sHLA-G) during CMV infection in maternal blood and amniotic fluid samples.

HLA-G is a non-classical HLA class I antigen characterised by a low allelic polymorphism, compared with the HLA class[21,22]. The HLA-G antigen is a tolerogenic molecule that acts on cells of both innate and adaptive immunity[23,24]. Interestingly, HLA-G expression by cytotrophoblasts is down-modulated by CMV infection[25], while it is up-modulated in peripheral blood cells, with possible functional consequences in pregnancy immuno-regulation[26,27].

In this study, sHLA-G levels in serum and amniotic fluid samples were assayed in triplicate as previously reported[28,29], using an enzyme-linked immunosorbent assay (ELISA) and the monoclonal antibody MEM-G9 (Exbio), which recognises HLA-G molecules, in β2-microglobulin associated form. The intra-assay coefficient of variation (CV) was 1.4% and the inter-assay CV was 4.0%; the limit of sensitivity was 1.0 ng/mL.

We have an interim analysis of a clinical prospective trial which is enrolling 400 pregnant women suspected at routine CMV testing to have active CMV infection. Here, we report the results obtained from a first cohort of 166 pregnant women. At the moment of serological-virological advanced diagnosis, we evaluated sHLA-G levels in 171 serum samples of 55 pregnant women with primary CMV infection, 31 with non-primary, 69 with past infection, and 11 CMV-uninfected. The median levels of sHLA-G in pregnant women with primary were higher in comparison with non-primary CMV infection (45.16 ng/mL *v.* 10.58 ng/mL, $P = 0.005$; Student's *t*-test). Furthermore, we observed lower median levels of sHLA-G serum between past CMV infected and uninfected women (14.68 ng/mL and 6.71 ng/mL, respectively). When we analysed the levels of sHLA-G in plasma samples from 55 primarily infected pregnant, considering transmitter and non-transmitter mothers, we did not find any statistical correlation ($P = 0.72$; Student's *t*-test).

Finally, we analysed 25 amniotic fluid samples collected during amniocentesis (20–21 weeks gestation)[19] from pregnant women with primary CMV infection arising before 14 weeks gestation. The comparison of the levels of sHLA-G between transmitter and non-transmitter mother was not statistically significant ($P = 0.38$; Student's *t*-test).

The limited sample size does not permit firm conclusions, however our preliminary results suggest that sHLA-G detected in maternal plasma samples might be an additional biomarker of CMV infection that could be considered in combination with currently used serological and virological markers.

Conclusion

The laboratory diagnosis of CMV infection proves to be a reliable tool, provided that pregnant women are checked from the first weeks of gestation. Moreover, the use of advanced serological and virological maternal tests allow clinicians to identify women who are at higher risk of transmitting CMV to their fetus; however, they do not identify the infected fetuses, therefore making it necessary to offer prenatal diagnosis.

Nevertheless, major limitations of prenatal diagnosis of CMV should be acknowledged; amniocentesis is an invasive procedure and positive results of amniotic fluid tests do not discriminate between infected fetuses and compromised fetuses. For this reason, researchers continue to work on the prognosis factors for the CMV disease. All in all, our very preliminary results in this study suggest that sHLA-G could be a sensitive marker in order to monitor CMV infection during pregnancy.

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Cytomegalovirus infection and pathogenesis in the human placenta



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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[1–3]. In Australia, 57% of women are seronegative and at risk for primary infection and transmission of virus to the fetus during pregnancy[4]. 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[5–8] and stillbirth[6,9,10]. 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[2]. 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.

Research on congenital HCMV infection has been hindered by the strict species specificity of the human virus. No animal model recapitulates the development and architecture of the human placenta, a hematogenous organ that survives by maternal tolerance of the fetal hemiallograft and performs critical functions throughout pregnancy. Currently, diagnostic indicators for primary and recurrent maternal HCMV infection have not been identified, nor are there any accepted treatments to prevent transmission. Development of a vaccine is in the early stages, long delayed by a poor understanding of the parameters of immune protection and routes of virus spread across the placenta[9,11–13].

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 *a*1). The individual chorionic villus contains a connective core with blood vessels that carry substances to the fetal circulation (panel *a*2). Placentation is a stepwise process whereby villus cytotrophoblasts (vCTB) attached to the basement membrane as a polarised epithelium leave the membrane to differentiate along one of two independent pathways depending on their location. In floating villi, they fuse to form a multinucleate synctiotrophoblast (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).

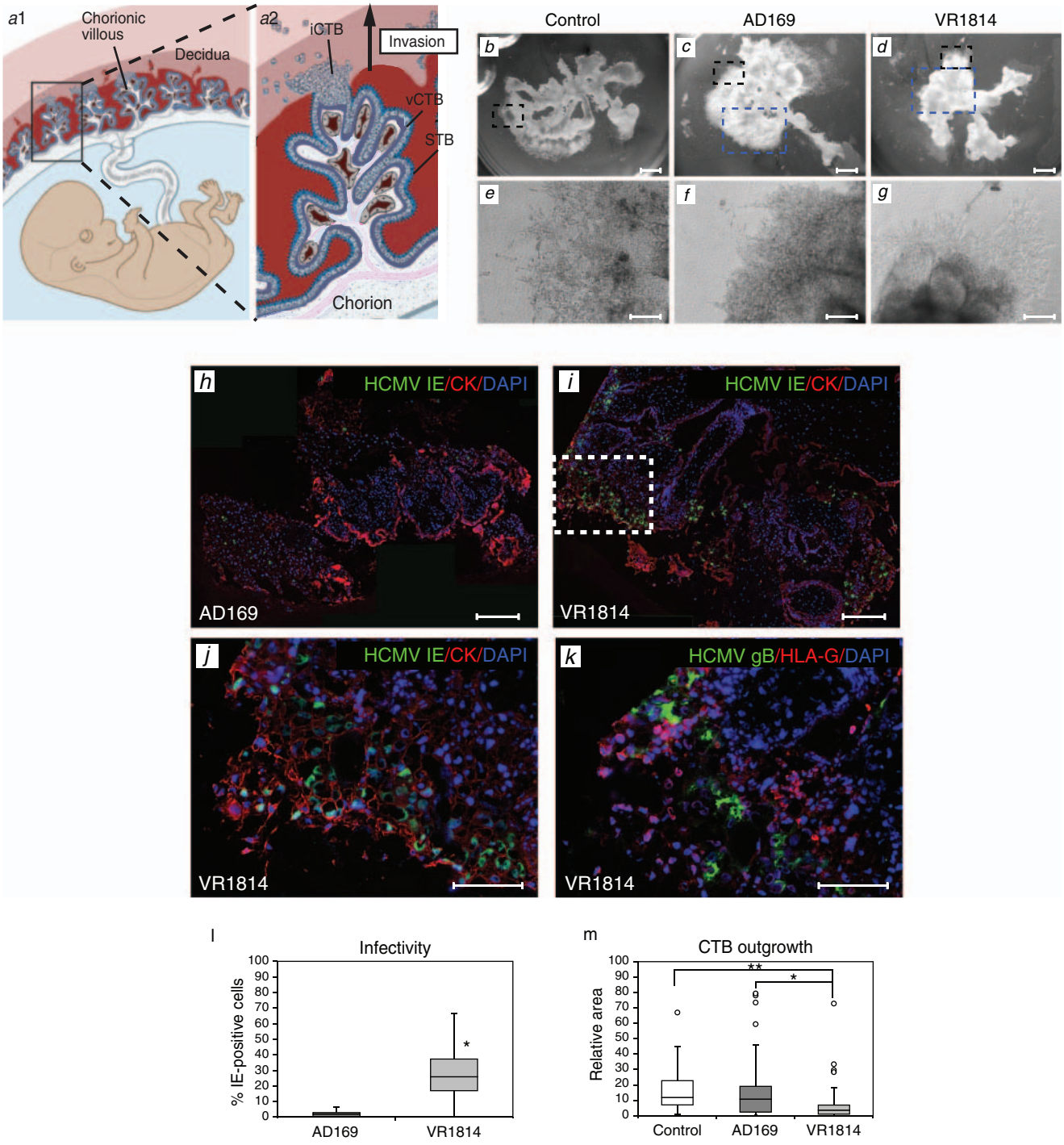

Figure 1. Anatomy of the human placenta and model for HCMV infection of villous explants *ex vivo*. Uterine-placental interface (*a*1–2). Chorionic villi, the functional units of the placenta, consist of syncytiotrophoblasts (STBs) covering the villous surface and villous cytotrophoblasts (vCTB), which terminally differentiate into invasive cytotrophoblasts (iCTBs) to form anchoring villi. Adapted from Maltepe *et al*.[14]. Villous explants infected with attenuated (AD169) and pathogenic (clinical) HCMV strains (VR1814), respectively. (*b–g*) Villous explants with anchoring villi (2x) and (*h*,*i*) insets (black dashed box, 100x). (*b*,*e*) Mock-infected controls. (*c*,*f*) AD169 infection. (*d*,*g*) VR1814 infection. Cytotrophoblasts immunostained for expression of cytokeratin 7 (CK) (red) and HCMV IE1&2 proteins (green) in explants infected with (*h*) AD169 (blue dashed box from *c*) and (*i*) VR1814 (blue dashed box from *d*). Original magnification x40. (*j*) Section from inset *i*, stained for CK and HCMV IE1&2 proteins (x200). (*k*) Adjacent section of *j* stained for HLA-G and HCMV gB (x200). Nuclei were counterstained with DAPI. (*l*) Number of AD169- and VR1814-infected cytotrophoblasts in cell columns. AD169 (explants = 5, cell columns = 19), VR1814 (explants = 6, cell columns = 33) $P < 0.0001$. (*m*) Measurements of anchoring villi of mock-infected control and AD169- and VR1814-infected explants. Mock-infected control (explants = 7, counted villi = 25), AD169 (explants = 7, counted villi = 21), VR1814 (explants = 16, counted villi = 43), $^{*}P < 0.001$, $^{**}P < 0.0001$ (Student's *t*-test).

Our studies of HCMV infection in the human placenta revealed important differences in infection between cell types[15–19] and maternal immune status[7,8,16,20]. Studies of primary human placental cells and tissue models have identified molecular pathways that impair trophoblast differentiation[17–19,21–24]. Patterns of viral proteins in infected placentas suggest modulation of infection in early

gestation[5,25–27] and formation of structural defects during pregnancy[7,8,18,23].

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[7,8,20,28]. 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[7,8].

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[29]. 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 (Figure 1*b*, *e*). Surprisingly, explants infected with the attenuated strain formed normal-size anchoring villi indistinguishable from controls (Figure 1*c*, *f*). 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 (Figure 1*d*, *g*). 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 (Figure 1*h*) 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 (Figure 1*i* inset, *j*). At late times, gB was made confirming viral replication and HLA-G was down-regulated (Figure 1*k*), suggesting infected cells could become targets of natural killer cells in the decidua[30].

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 (Figure 1*l*). 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 1*m*). 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[10]. Our immunohistological 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[29]. 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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Vaccination for cytomegalovirus: when, where and how

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Although following primary human cytomegalovirus (CMV) infection in many individuals no overt symptoms are observed, CMV came to medical attention due to its significant morbidity and mortality associated with congenital infection and immunosuppressed individuals. Congenital infection occurs following transplacental transmission during pregnancy as a result of primary infection, reactivation or re-infection with a different isolate. Estimates suggest at least a million cases of congenital CMV occur annually worldwide. Congenital infection is a leading cause of neurological complications such as mental retardation, cerebral palsy, developmental delay and seizure disorders and also causes permanent disabilities, such as hearing loss and vision impairment. In addition, other common manifestation of CMV infection are stillbirth, preterm delivery and intrauterine growth restriction (IUGR) and cardiovascular disease, which are risk factors for perinatal and lifetime morbidity. Recent reports have estimated that the economic costs to public health and families due to congenital CMV infection are immense, with direct annual costs of billions of dollars. An effective CMV vaccine that could prevent transplacental transmission, reduce CMV disease and CMV-associated stillbirths has been recognised as an urgent medical need. Over the past 40 years several CMV vaccine candidates have been evaluated in a series of clinical trials and found to be effective in preclinical and clinical studies. However, in spite of extensive efforts over many decades, successful licensure of an effective CMV vaccine formulation to prevent congenital CMV infection remains elusive.

The major target populations for a CMV vaccine to reduce congenital infection include women of reproductive age, infants, toddlers and adolescents. Children who attend day care represent a particularly important reservoir of CMV. Women of reproductive age exposed to children who are shedding virus in urine and saliva are 10 times more likely to seroconvert compared to women unexposed to virus shedding children[1,2]. In addition, the symptomatic congenital CMV rate is very high if women acquire primary infection or CMV virus reactivates during or just before pregnancy, whilst prior natural CMV infection provides protection from transplacental transmission[3–6]. In pregnant women following natural primary infection, viral replication is controlled by the emergence of antigen-specific $CD4^+$, $CD8^+$ and $CD45RA^+$ effector memory T-cells[7,8]. Lower frequencies of CMV-specific $CD4^+$ T-cells and $CD45RA^+$ cells in mothers following primary infection is known to be associated with virus transmission to the fetus[9,10]. Based on these observations it can be hypothesised that emergence of higher frequencies of CMV-specific $CD4^+$, $CD8^+$ and $CD45RA^+$ effector memory T-cells may be associated with control of viremia and prevention of transplacental transmission. Therefore, an effective CMV vaccine specially designed to induce CMV-specific $CD4^+$, $CD8^+$ and $CD45RA^+$ effector memory T-cells in women of reproductive age could potentially decrease CMV transmission to the fetus and vaccination of infants, toddlers and adolescents could reduce the duration of viral shedding, which may reduce child-to-mother transmission.

Clinical evaluation of active and passive immunisation strategies against CMV in the context of congenital CMV

Live attenuated virus vaccines

The initial CMV vaccine trials based on an attenuated form of CMV isolates Towne and AD169[11,12] induced humoral and cellular

immune responses in immunocompromised solid organ transplant patients. However, a study conducted in young women with children attending group day care showed no reduction in the infection rate in Towne-vaccinated mothers compared to placebo. Thus the efficacy of the Towne vaccine against congenital CMV infection was questioned[13]. Subsequently several alternative approaches have been used to improve the efficacy of the live attenuated Towne vaccine, which includes generating recombinant chimeras by swapping lost genome segments of Towne vaccine with the less attenuated Toledo strain. The evaluation of safety and immunogenicity of chimeras in CMV-naïve subjects is now in phase 1 clinical trials[14]. However, safety concerns raised by experts in the field during an FDA review in 1999 are the major confounders to vaccination with any live attenuated CMV vaccine[14].

Subunit vaccines

Another vaccine strategy is based on a subunit vaccine that was developed initially by Chiron by combining recombinant glycoprotein B (gB) with an oil-in-water adjuvant, MF59. The efficacy of gB-MF59 vaccine was recently evaluated in a Phase II, double-blind, randomised, placebo-controlled trial, in seronegative women of child bearing age[15]. The vaccine showed reduction in the incidence of primary maternal infection by 50% in the vaccinated group compared to the placebo group. However, the protection was not durable and it was predominantly observed within the first year after immunisation. Subsequent testing of gB-MF59 vaccine in seropositive women showed a significant boost in neutralising antibody titers and $CD4^+$ T-cell responses[16]. Nevertheless, whether such boosting will provide protection against reactivation or reinfection with a different isolate in women with pre-existing immunity is not yet known.

Passive immunisation

In addition to active immunisation strategies, passive immunisation strategies based on administration of anti-CMV immune globulin to women at high risk of transmitting CMV to the fetus also have been explored in clinical research. Initial observations suggest that CMV hyperimmunoglobulin (HIG) could inhibit viral spread *in vitro*[17,18], restore placental health in mothers during primary infection[19] and lead to the regression of fetal cerebral ultrasound abnormalities[20]. A prospective study carried out in mothers with confirmed primary infection demonstrated that monthly intravenous administration of HIG can decrease mother-to-fetus transmission significantly, from 40% to 16% and the risk of congenital disease from 50% to 3%[21]. Several retrospective studies have suggested that CMV HIG can reduce intrauterine transmission of CMV[22] and can protect against poor outcomes in infants[23,24]. However, in a recent randomised trial, HIG treatment did not significantly modify the course of primary CMV infection during the pregnancy[25]. Therefore, in our perspective more randomised studies are required to draw a firm conclusion on the efficacy of HIG therapy.

CMV vaccines in preclinical studies

Several additional proof-of-concept studies of various candidate vaccines have also been evaluated in clinical and preclinical studies in recent years[14,26]. Vical/Astellas developed a vaccine (CyMVectin™) to target congenital CMV using plasmids that encode gB and pp65 formulated with Vaxfectin adjuvant. The preclinical data from this study presented at the 5th International Congenital CMV Conference (2015) held in Brisbane showed that CyMVectin™ has the potential to induce neutralising antibodies against both fibroblasts and epithelial cells. AplhaVax developed a dual alphavirus replicon that expresses the CMV antigens gB plus an IE1-pp65 fusion protein and has evaluated its safety and immunogenicity in Phase I clinical trials. Following vaccination all vaccine recipients developed polyfunctional $CD4^+$ and $CD8^+$ T-cell responses and neutralising antibody response[27,28]. Furthermore, several alternative vaccine strategies have shown promising results in emerging preclinical evaluations[26]. These include a recombinant modified vaccinia Ankara (MVA) expressing three immunodominant CMV antigens pp65, IE1 and IE2 as a fusion protein, a dense body vaccine consisting of non-infectious, replication-defective particles formed during the replication of CMV, polyepitope vaccines comprising a replication-deficient adenoviral vector for the expression of gB antigenic domain-1 or the extracellular domain of the gB protein and 46 HLA class I and II restricted T-cell epitopes or recombinant gB and polyepitope protein formulated with TLR4 and TLR9 agonists[29]. However, the safety and immunogenicity of these vaccine candidates is yet to be determined in advanced clinical studies in the context of congenital CMV.

Extensive studies in humans have revealed that the gH/gL/UL128–131A pentameric complex is the most important antigenic complex for neutralising antibodies especially to restrict the entry of CMV into epithelial and endothelial cells[30]. High titers of neutralising antibodies are thought to protect against transmission by blocking receptor-mediated transplacental transmission of CMV and by reducing viral replication[31,32]. Therefore, it will be interesting to investigate the potential role of pentameric glycoprotein complex-specific humoral responses in both primary and recurrent infections in pregnant women.

Major barriers in the development of effective CMV vaccines

Conventional CMV vaccine approaches that target a single genotype may induce only partial protection due to high levels of CMV

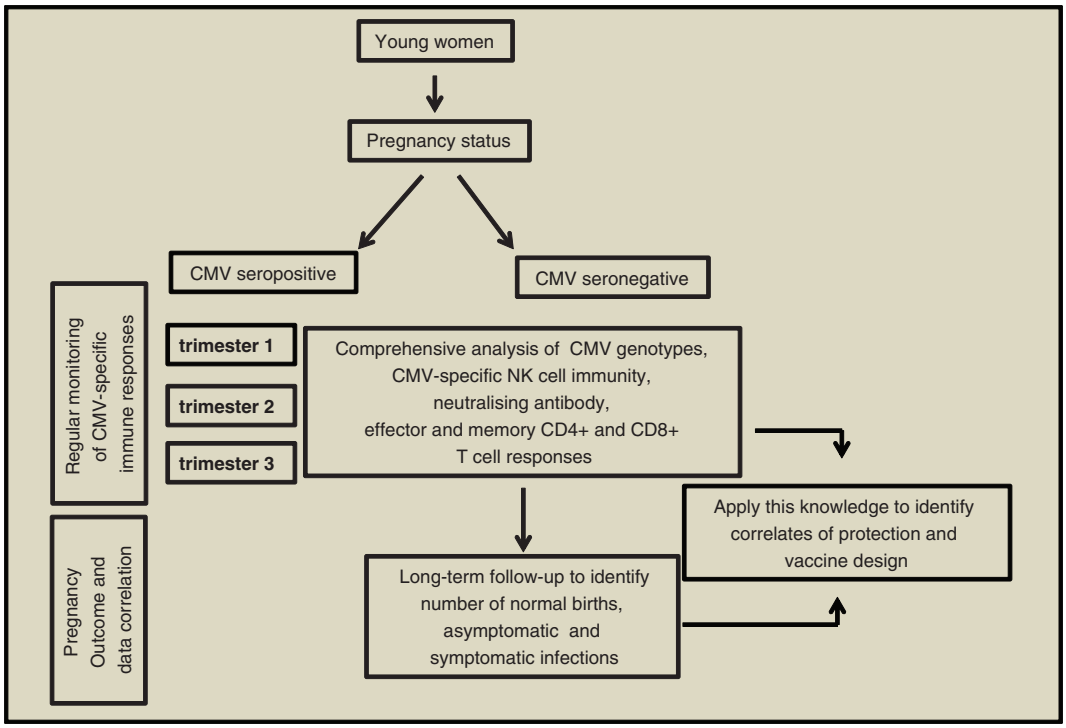

Figure 1. Proposed method for screening and understanding the magnitude of protective immune responses during pregnancy. A large cohort of young women before and after pregnancy should be screened for cytomegalovirus (CMV) status. Regular monitoring of CMV-specific immune responses should be carried out using several comprehensive analysis approaches and pregnancy outcomes after long-term follow-up should be related to the magnitude of the immune response observed during the study. This knowledge can be used for the development of a potent CMV vaccine.

genomic variation and recombination within infected populations[33,34]. Frequent recurrence and transmission in largely CMV seropositive individuals are the major confounders for CMV vaccine development. Despite the promising results from CMV-HIG trials, the mode of action of these antibodies in limiting transplacental transmission of CMV in high-seroprevalence population remains to be determined. Finally, understanding the immune parameters that effectively protect from transplacental transmission of CMV in pregnant women as a result of primary infection, reactivation or re-infection need to be delineated for the development of an effective CMV vaccine (Figure 1).

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An effective and feasible approach to prevention of primary cytomegalovirus infection in pregnancy





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In the absence of a cytomegalovirus (CMV) vaccine, other strategies for prevention of primary infection in pregnancy should be considered. Behavioural interventions have been reported to significantly decrease seroconversion rate among seronegative pregnant women. We report here on a recently completed controlled study in which seronegative women at high risk of infection because of close contacts with children <36 months, were identified and informed about risky and protective behaviours. Informed women seroconverted at a significantly lower rate than non-informed women.

When in 1974 Elek and Stern published their paper with the committing title 'Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero'[1] they could not imagine that 40 years later the CMV vaccine would still be an elusive target, and the hope that '. . . the use of such a vaccine in adolescent girls would reduce the incidence of primary cytomegalovirus infection in pregnancy and thus eliminate fetal brain damage due to this cause' would remain unmet. Although considered top priority[2] and highly desirable[3], the availability of a CMV vaccine is not foreseen for the near future. Sadly, nowadays, seronegative pregnant women are at the same risk of acquiring a primary CMV infection as they were decades ago. Congenital CMV is perpetuated by the lack of public awareness, serologic screening, unavailability of an effective vaccine and of effective therapeutic treatments.

On the other hand, our knowledge about the epidemiology of the virus and its ways of transmission, together with the availability of reliable serology assays have the potential, when properly used, to markedly reduce the incidence of primary infection in pregnant women and, ultimately, of congenital infection.

In two small studies conducted in Virginia, US, in 1996 and 2004 the group of S. Adler reported that: (1) behavioural interventions such as frequent hand washing and avoiding close contacts with young children had the potential of reducing the risk of primary CMV infection; and (2) pregnant women were more motivated to follow behavioural interventions than were nonpregnant women[4,5]. In other words, four conditions had to be satisfied for hygiene recommendations to be effective, namely the woman had: (1) to be pregnant; (2) to

know to be at risk (i.e. seronegative); (3) to be informed about hygiene measures; and (4) to comply with behavioural recommendations. Although the above studies were not powered to answer the key questions and suffered from substantial methodological limitations[6], they were important pilot studies and they were instrumental to the design of subsequent larger studies. More recently, a French study conducted among more than 5000 pregnant women at a single hospital in Paris showed a reduction from 0.4% to 0.2% in the seroconversion rate following CMV counseling[7]. However, the control group appeared questionable from the methodological point of view because seroconversions diagnosed prospectively in the informed group in the second and third trimester of gestation, were compared to primary infections retrospectively diagnosed as having occurred in the first trimester (0–12 weeks' gestation) on the basis of presence of IgM and IgG avidity results. Behavioural interventions have been reported to be highly successful for the prevention of other infectious diseases transmitted via contaminated hands[8]. Presently, however, quite a degree of uncertainty remains that a behavioural intervention can effectively encourage pregnant women to consistently follow CMV recommendations[9].

Inspired by the above studies, we designed a controlled study to investigate the actual effectiveness and feasibility of an intervention, based on: (1) identification of seronegative pregnant women at high risk of primary infection (i.e. having frequent contacts with children <36 months because of personal or occupational reasons); and (2) CMV counseling about risky and protective behaviours to reduce the risk of primary infection. The study is reported in detail elsewhere[10]. Briefly, the study comprised an intervention arm and an observational control group. In the intervention arm, CMV-seronegative women, identified at the maternal serum screening for foetal aneuploidy at 11–12 weeks' gestation, were given hygiene information and prospectively tested for CMV until delivery. Suggested hygiene measures included frequent hand washing after exposure to children's bodily fluids, frequent washing of surfaces touched by the child (toys, high chair, stroller, etc.), avoid kissing the child on the mouth/cheeks, and sharing utensils, washcloths, food or drink, as well as putting in the mouth whatever may have been in the child's mouth. The control arm consisted of women enrolled at delivery who were neither tested for, nor informed about CMV during pregnancy, and who had a serum sample stored at the time screening for foetal aneuploidy. The primary outcome was CMV seroconversion.

Four out of 331 (1.2%) women seroconverted in the intervention group compared to 24 out of 315 (7.6%) in the control group ($\Delta = 6.4\%$; 95% CI 3.2–9.6; $P < 0.001$) (Figure 1). There were three newborns with congenital infection in the intervention group and 8 in the control group (one with cerebral ultrasound abnormalities at birth). Of the four seroconverting mothers in the intervention group, one was most likely already infected at the time of enrolment, one reported sleeping in the same bed with her sick child, one reportedly followed recommendations, while the last one reported scepticism about hygiene measures since enrolment. Apparently, her scepticism was shared by her obstetrician as well. Apart from the latter case, the study was generally well accepted by the obstetricians caring for the participants.

As for acceptance of, and compliance to recommendations, 93% of women felt hygiene recommendations were worth suggesting to all pregnant women at risk for infection, and 80% reported substantial or complete adherence to hygiene recommendations.

In conclusion, we hope that in view of the strikingly positive results obtained in our study, the view point of some that 'Currently, there

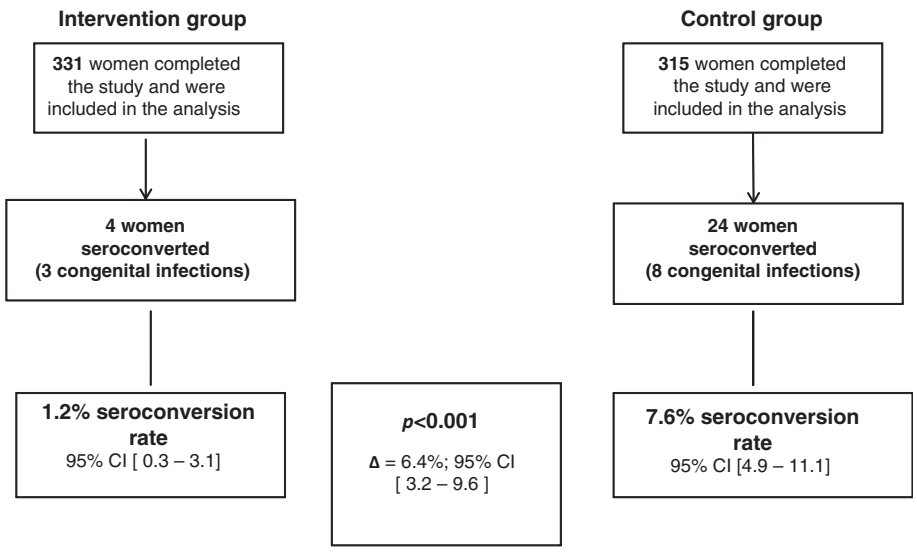

Figure 1. Seroconversion rate in the intervention and in the control group. Seroconversion rate among women who did not receive cytomegalovirus (CMV) information rises to 9% when nine women diagnosed with a recent primary CMV infection at first testing at 12 weeks gestation are included.

are inadequate data to show that education actually changes behaviour and that this behaviour change translates into decreasing maternal infection and subsequent congenital infection'[11] will be revised in the near future. Clearly, there are still many obstacles ahead, such as poor training of health professionals in behavioural counselling, and some scepticism among obstetricians about the effectiveness of behavioural intervention. Unfortunately, the latter is sometimes shared by patients too. Indeed, both categories (i.e. physicians and patients) are keener to consider solving health problems by means of a medical act (drug, surgery) rather than through a modification of behaviour which is certainly more time-consuming and demanding (on a personal level) to achieve. Additionally, an updated cost-benefit analysis of a behavioural intervention needs to be performed which, in turn, raises the thorny and complex issue of prenatal CMV screening. Nevertheless, we believe that the reported feasibility and effectiveness of a primary prevention intervention will help, in the first instance, by providing evidence-based data. Strong experimental data together with the identification of the best channels to reach health professionals and pregnant (childbearing) women and to deliver the most appropriate health messages will eventually lower barriers to the implementation of behavioural prevention strategies.

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HIV in pregnant women and prevention of perinatal transmission

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Women with HIV who have access to treatment can expect to have a normal life expectancy. With effective antiretroviral therapy, an undetectable viral load, and avoidance of breast-feeding, the rate of perinatal transmission is extremely low (<1%). A Caesarean section is no longer routinely recommended nor is intrapartum zidovudine. Women living with HIV should be supported in their decision regarding parenthood given their excellent prognosis, low risk of perinatal transmission and reproductive rights. If interventions to reduce perinatal HIV transmission during pregnancy and post-partum are embraced, women can expect to have an uninfected infant.

This article provides a succinct review of women, HIV and pregnancy and is focused on the resource rich setting. Differences exist in management and access to interventions in the resource-poor setting but this will not be discussed in this manuscript.

Women with HIV who have access to lifelong combination antiretroviral therapy can expect to have a normal life expectancy. This combined with extremely low rates of perinatal transmission has resulted in many women now contemplating plans for parenthood. In addition, the risk of HIV transmission to their partner through the process of conception can be significantly reduced by use of antiretroviral therapy (by the HIV infected woman) or by pre exposure prophylaxis (by the uninfected male partner) if they choose to conceive via condomless sex. Alternatively self-insemination can be utilised for conception if the HIV infected partner is the woman, posing no risk of HIV transmission to the uninfected partner, or assisted reproduction may be utilised if there are fertility issues. Once pregnant, the risk of perinatal transmission can be reduced to <1% if the woman takes combination antiretroviral therapy during the pregnancy and the neonate has four weeks of antiretroviral monotherapy, combined with avoidance of breast-feeding. A Caesarean section is no longer routinely recommended nor is intrapartum intravenous zidovudine.

Conception

The prognosis of adults living with HIV has continued to improve in the past two decades with the use of combination antiretroviral therapy. Mathematical modelling now suggests that for an adult newly diagnosed with HIV in a resource rich setting, with access to lifelong medication, life expectancy approaches that of HIV uninfected adults[1]. This combined with the extremely low rates of perinatal transmission reported[2] many women living with HIV are contemplating their options for parenthood. One important step in this decision-making is the optimal and safest method of conception. Options for conception need to take into account the HIV status of the partner and fertility issues. Women who are infected with HIV have the option of condomless sex with their partner, self-insemination or assisted reproduction depending on availability and any existing fertility issues. The most important intervention to reduce transmission of HIV to the uninfected partner is treatment with antiretroviral therapy, either as treatment for prevention to the HIV infected partner[3] or as pre exposure prophylaxis (PrEP) in the HIV-uninfected partner[4,5]. Data regarding the use of PrEP for conception are limited to observational studies[6].

Management during pregnancy

The most important intervention to reduce perinatal HIV transmission is antiretroviral therapy for the mother[7]. It has been well established that combination therapy (rather than monotherapy) is the most effective method for reducing maternal viral load, the most important predictor of perinatal transmission[8]. Recent data suggest that the earlier treatment is started, the lower the transmission rate[2] and if women enter pregnancy already on antiretrovirals then these should be continued[2]. Although safety data are still lacking on some of the newer antiretrovirals, the antiretroviral pregnancy registry provides reassuring data excluding teratogenicity for the majority of prescribed first line antiretrovirals[9].

Historically, elective Caesarean section was recommended as an intervention to reduce perinatal transmission, although the data to support this were primarily before the advent of combination therapy with more recent analyses suggesting the risk of perinatal HIV transmission is comparable in women with an undetectable viral load[10,11]. Today, vaginal birth is the recommended mode of delivery in women with an undetectable viral load (defined as either <50 copies/mL in guidelines from the United Kingdom to <1000 copies/mL in guidelines from the United States)[12,13] unless there is an obstetric indication for a Caesarean section. Similarly, intrapartum zidovudine was previously prescribed routinely but is now reserved for women with a viral load at the time of delivery of >1000 copies/mL[12,13]. In addition to antiretrovirals for the mother, it is recommended that exposed neonates receive four weeks of monotherapy to further reduce the risk of HIV transmission.

Breastfeeding

When considering breastfeeding, the individual setting needs to be taken into account as guidelines regarding breastfeeding differ significantly between resource rich and resource poor settings. HIV has been found in breast milk and the only way to guarantee that HIV will not be transmitted after birth is for complete avoidance of breastfeeding. However when this is not possible or safe, recent studies have confirmed that with the use of antiretroviral therapy to either the mother or the infant for the duration of breastfeeding, rates of transmission can be reduced[14–16]. This has re-ignited debate regarding the support of women especially in resource rich settings who desire to breast feed despite the availability of safe, affordable, culturally appropriate alternatives. In this setting, although the recommendation remains avoidance of breastfeeding, it is becomingly increasingly recognised that if a well informed woman elects to breastfeed, knowing the potential risk associated with this, that she should be supported in this decision making process to maximize the likelihood of adherence to antiretroviral therapy rather than it be seen as a child protection issue[17].

Management of the neonate

It is essential that infants born to HIV infected mothers are appropriately followed up and tested after birth. The options for testing include a p24 antigen, HIV RNA, and/or an HIV proviral DNA. The choice of test depends on local availability. For diagnosis of HIV in the neonate, all these tests are suitable and appropriate. The most important issue is that the infant has follow up testing, rather than which test is available, given the equivalent performance of these tests in experienced hands. The recommended timing of testing also varies depending on the individual setting but commonly accepted protocols for follow up testing includes testing in the first week of life, at six weeks, and then three months provided no ongoing exposure via breastfeeding occurs.

Conclusion

Women living with HIV should be supported in their decision regarding parenthood given their excellent prognosis, low risk of perinatal transmission and reproductive rights. Importantly however, these women need to be well informed regarding the interventions available to reduce risk of HIV transmission to their partners (if they are HIV negative) and to their infant. If these are embraced women can have normal vaginal births and expect to have an uninfected infant.

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Biography

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Diagnosis of congenital syphilis and toxoplasmosis

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Syphilis, toxoplasmosis, and cytomegalovirus represent disparate entities. The bacterial spirochaete *Treponema pallidum* ssp. *pallidum* causes syphilis, the 'The Great Imitator'; the organism's sole natural host is humans and it remains exquisitely sensitive to penicillin. By contrast, the zoonotic parasite *Toxoplasma gondii* causes toxoplasmosis. Infection is usually self-limited, although serious disease can occur in the immunocompromised. Meanwhile, the human cytomegalovirus (CMV; human herpesvirus 5) is a relatively prevalent enveloped DNA betaherpesvirus with infection specific to humans. Despite nomenclatural, ecological and therapeutic disparities, however, these agents exhibit several concordances, including various, and at times, cryptic syndromes in child and often mother; congenital infections with potentially devastating outcomes; diagnostic dilemmas. This article primarily discusses the latter of these issues in relationship to congenital syphilis and toxoplasmosis in the Australian context.

Syphilis

The number of cases of congenital syphilis has fallen in Australia over the past two decades (1995–2004: 146 cases; 2005–2014: 64 cases)[1]. However, there has been a recent resurgence of syphilis particularly amongst men who have sex with men[2], with the overall incidence of infectious syphilis of less than 2 years duration more than doubling (2004–2006: mean 3.5/100 000; 2012–2014: mean 7.7/100 000)[1]. Additionally, the burden of disease in indigenous populations is well recognised, and it is possible that fly-in fly-out workers could transmit infections[3]. In the United States and United Kingdom re-emergence is also underway, and congenital infections have been linked particularly to primary and secondary syphilis in females[4–6]. Given the potential for further spread in Australia, ongoing vigilance is required.

Vertical transmission is highest in primary and secondary syphilis, continuing throughout pregnancy while the severity of outcomes decreases[7]. Adverse outcomes can include stillbirth or miscarriage, perinatal death, prematurity, low birthweight, and a series of early congenital manifestations (e.g. snuffles, hepatosplenomegaly, generalised lymphadenopathy, bony lesions) and late congenital manifestations (typically due to chronic granulomatous inflammation)[4,8,9].

In Australia, universal screening for syphilis is recommended in pregnancy, with a treponemal-specific serological assay performed at the first antenatal visit, and repeat screening in high-risk populations should be considered at 28 weeks[10,11]. Modalities available for diagnosing congenital syphilis in Australia include both treponemal-specific and non-treponemal specific serology, nucleic acid detection, direct fluorescent antibody testing, and histochemical staining (e.g. Warthin-Starry silver stain) (Table 1); methods of historical

Table 1. Methods available for the diagnosis of congenital syphilis in Australia.

Method (Sample)	Description	Approximated performance (see[12])
Nucleic acid detection		
Polymerase Chain Reaction (Various)	Amplify *T. pallidum* specific sequences (e.g. 47-kDa protein gene, and DNA polymerase I gene)	Performance varies with sample type and assay Sensitivity typically >80% Specificity >97%
Treponemal specific serology: *For detecting past or active syphilis. Reactivity may not necessarily indicate active infection*		
Syphilis EIA or CMIA (e.g. Abbott Architect Syphilis TP) (Serum, Plasma)	Use recombinant antigens (e.g. TpN15, TpN17, TpN47) and anti-human immunoglobulin conjugate. Typically suitable for automation	Sensitivity > 99% Specificity > 99%
Fluorescent *T. pallidum* Antibody Absorption (FTA-Abs) Test (Serum, CSF)	Indirect immunofluorescence slide test based on Nichols strain *T. pallidum*, non-pathogenic Reiter treponeme sorbent, and anti-human IgG conjugate	Sensitivity: 86% (primary), 100% (secondary), 98% (latent), 73% (late) Specificity: 94–100%
T. pallidum Particle Agglutination (TPPA) (Serum, Plasma)	Gelatin particle agglutination coated in antigen from Nichols Strain *T. pallidum* (an alternative is the *T. pallidum* haemagglutination assay (TPHA) using erythrocytes)	Sensitivity: > 99% Specificity: > 99%
T. pallidum IgM EIA (Serum)	Anti-human IgM antibodies capture IgM and are incubated with a purified *T. pallidum* antigen	Sensitivity: > 86.5% (primary), lower later Specificity: 90%
Non-treponemal specific serology: *For detecting or monitoring active infection, but nonspecific to syphilis*		
Rapid Plasma Reagin (RPR) (Serum, Plasma, +/− CSF)	A macroscopic flocculation test using reagin binding to cardiolipin-lecithin-coated cholesterol particles	Sensitivity: 86% (primary), 100% (secondary), 98% (latent), 73% (late) Specificity: 93–98%
Venereal Diseases Research Laboratory (VDRL) Test (Serum, CSF)	A microscopic flocculation test using a liposomal suspension of stabilised cardiolipin	Sensitivity: 78% (primary), 100% (secondary), 95% (latent), 71% (late) Specificity: 98–100%
Others		
Direct Fluorescent Antibody Test (DFA-TP) (e.g. lesion smear)	Fluorescein isothiocyanate-labelled antibody attaches to treponemal antigen	Sensitivity and specificity higher than dark field microscopy. Dependant on stage and sample
Silver stains (e.g. tissue)	Stains organisms dark brown to black on a light golden background	Performance variable. Dependant on stage and sample

CMIA, chemiluminescent microparticle immunoassay; CSF, cerebrospinal fluid; EIA, enzyme immunoassay.

interest include dark field microscopy and *in vivo* culture using rabbit testes. Test selection and interpretation is complicated by the variety of tests available, with performance characteristics varying with disease progression. The Public Health Laboratory Network (PHLN) laboratory case definitions provide definitive and suggestive criteria for congenital, acquired active, and acquired active re-infective syphilis[12]; these criteria define testing strategies and complement the 'Syphilis (congenital) case definition' published by the Communicable Diseases Network Australia[13].

It is beyond the scope of this article to detail the interpretation and implications of the gamut of serological profiles potentially observed in maternal and congenital syphilis. Instead, the reader is referred to diagnostic algorithms and case definitions published elsewhere (e.g.[12–14]). When investigating potential congenital syphilis, areas of consideration include:

- Undertaking a rigorous history including exposure history, treatment history and its adequacy, and assessment of factors that predispose to false positive and negative results such as pregnancy and auto-immune disease.
- Undertaking a rigorous examination of mother and infant.
- Reviewing maternal and newborn serology. For example, assessing maternal or newborn syphilis titres for significant increases, or for newborn titres that are 4-fold greater than maternal titres.
- Awareness of the performance of non-treponemal serological tests (see Table 1), and reasons for misleading results (Table 2).

Table 2. Factors potentially contributing to missed or incorrectly diagnosed maternal syphilis during screening.

Reasons for missing a maternal syphilis diagnosis	
1	Mother newly acquires syphilis after antenatal screening
2	Mother is reinfected during pregnancy, which is misinterpreted as past infection during antenatal screening
3	Mother acquires syphilis before antenatal screening but does not seroconvert until after screening
4	Mother has inadequately treated prior syphilis, with potential suppression of antibody
5	Undiagnosed prior infection partly treated with therapy for an alternative condition suppressing antibody
6	Mother has a longstanding infection with natural decline in antibodies
7	A test-specific false negative occurs
8	Mother is immunosuppressed (e.g. HIV) with a modified serological response
9	Failure to screen
10	Overt or occult pre-analytic, analytic or post-analytic error
Reasons for incorrectly diagnosing maternal syphilis	
1	Mother has adequately treated prior infection, with is misinterpreted as active infection
2	A test-specific false positive occurs
3	Overt or occult pre-analytic, analytic or post-analytic error

For example, have false positives (e.g. biological) and false negatives (e.g. potentially methodological such as prozone[15], or biological such as natural titre decline irrespective of therapy) been considered? Were separate serum specimens tested in parallel given inter-observer and inter-laboratory variation in endpoint determination[12]?

- Awareness of potential weaknesses in treponemal-specific serological tests (see Table 1) and reasons for misleading results (Table 2). For example, has a false negative in a very recent exposure (i.e. before seroconversion) been considered? Such consideration is particularly warranted when history identifies possible exposure to syphilis (e.g. an infected partner), or examination reveals a chancre-like lesion.
- Awareness of sample selection. As an example, umbilical cord blood samples may be less preferable than infant serum given maternal blood contamination could incorrectly suggest congenital infection[16].
- Awareness of sample management. As an example, recognition that seroreversion can occur with inappropriate storage conditions or freeze–thaw cycling (e.g. after repeated parallel testing)[17].
- Awareness of the utility of nucleic acid testing (NAT) testing. For example, confirmation of diagnosis may be achieved using paraffin-embedded tissue samples[12].
- Adequate follow-up. For example, testing of an infant might be considered if maternal diagnosis of syphilis occurs within one year of delivery[18].

Despite such considerations and well characterised testing pathways and definitions, and universally recommended testing, numerous factors could contribute to missed or incorrect diagnoses (Table 2). Suspicious or discordant cases may be evaluated through reviewing patient history, retesting (potentially with an alternate assay) and comparing results. Expert advice should be sought when interpreting discordant results in relationship to interpretive criteria. Additionally, all diagnoses of maternal or congenital syphilis require notification in Australia.

Toxoplasmosis

Like congenital syphilis, congenital toxoplasmosis is believed to be uncommon in Australia. Being non-notifiable, diagnostic records are not collated, and epidemiological analysis is challenging. Between 2001 and 2009, incidence rates of *T. gondii* based on seropositivity analysis were estimated at 0.017 per 1000 live births in New South Wales[19]. International estimates vary considerably[20], dependant on disease burden and methodology. While possibly fewer than 20 symptomatic congenital cases are diagnosed in Australia annually, the disease burden remains poorly understood particularly amongst indigenous and remote communities.

Disease occurs after maternal exposure to *T. gondii* via, for example, consumption of undercooked meat, or ingestion of oocyst contaminated soil and water[21,22]. The frequency of vertical transmission after exposure increases as pregnancy proceeds, while disease severity typically declines[23]. Although most cases are subclinical, manifestations can be broad-ranging with sequelae unrecognised for decades[23]. Outcomes may include spontaneous abortion, still birth or prematurity, or manifestations such as

Table 3. Examples of pitfalls associated with *Toxoplasma gondii* serology (e.g. see[30,31]).

Test	Use	Pitfall
IgG	May be used to identify current or past infection	May not be detected in first 2–3 weeks post exposure
		Some immunocompromised patients remain IgG negative
		Presence does not discriminate between recent and past infection
		Avidity testing helps discriminate early from late infection, although elevated avidity in late pregnancy may not exclude infection during early pregnancy
		May miss late infection subsequent to screening
		May be transiently negative in congenital cases due to maternal and neonatal treatment
		A positive result does not preclude possible reinfection
		May be positive in neonate at birth due to maternal IgG but not therefore indicative of congenital infection
IgM	May be used to identify more recent infection	May persist for up to 12 years post infection
		May be falsely positive in some assays due to rheumatoid factor or antinuclear antibodies
		May be negative in congenital cases where IgA positive
		May be negative in very early infection
		May miss late infection subsequent to screening
		May be transiently negative in congenital cases due to maternal and neonatal treatment
		Contaminating maternal antibodies may be detected in neonate despite no congenital infection, warranting retesting after delay
		Natural IgM antibodies may be present that do not signify infection
IgA	May be used to identify more recent infection	May persist post infection
		May miss late infection subsequent to screening
		May be negative in very early infection
		Variable period of seropositivity possible
		IgA versus IgM kinetics potentially differ in adults versus neonates
		Contaminating maternal antibodies may be detected in neonate despite no congenital infection, warranting retesting after delay
IgE	May be used to identify more recent infection	Duration of seropositivity typically shorter than IgA or IgM
		Duration of seropositivity can vary
		May miss late infection subsequent to screening

hydrocephalus, chorioretinitis, intracranial calcifications, jaundice, hepatomegaly, thrombocytopenia, cerebral spinal fluid abnormalities, and motor abnormalities[23,24].

The role of screening for toxoplasmosis has long been debated (e.g.[25]). The low incidence of toxoplasmosis in Australia translates to a low pre-test probability of infection. Combined with issues of

imperfect testing and the risks associated with potentially invasive confirmatory tests, routine antenatal screening for *T. gondii* infection is not recommended. Instead, pregnant women are presumed non-immune and advised to routinely avoid risk activities, although evidence supporting prenatal education as a method of preventing congenital toxoplasmosis remains sparse[26]. Similar approaches are recommended in other low prevalence countries (e.g. Canada[27]). Conversely, higher prevalence countries such as Denmark, France, Italy and Germany promote screening for congenital toxoplasmosis[28]. In Australia, selective testing is only recommended in women at 'substantially increased risk of infection'[10], although evidence based criteria are not available. Women whose history and examination indicates a substantially increased risk of infection, including those who are symptomatic, should be tested.

When required, diagnosis frequently relies on the interpretation of serological profiles. A typical approach might include assessment of IgG and IgM reactivity (e.g.[14,29]), whereby:

- IgM non-reactivity with IgG non-reactivity usually indicates no past or recent infection;
- IgM non-reactivity with IgG reactivity usually indicates past infection; and
- IgM reactivity with IgG reactivity or non-reactivity could indicate recent infection, prolonged IgM persistence or a falsely positive IgM.

Numerous pitfalls are associated with the interpretation of *Toxoplasma* serology (Table 3). Additionally, no unified Australian case definition for human toxoplasmosis exists, although definitions are available elsewhere[32,33]. Key considerations when diagnosing maternal toxoplasmosis include:

- Differentiating uninfected cases from those with IgM reactivity (e.g. when IgG is non-reactive and IgM reactive). Consider testing stored prenatal samples (if available), repeat testing after two weeks to identify seroconversion indicative of recent infection, and retesting the same specimen with an alternative IgM assay. If the IgG remains non-reactive, revisit possible reasons for the reactive IgM (Table 3).
- Differentiating recent infections from past infections (e.g. when both IgG and IgM are reactive). Approaches include IgG avidity testing[34], and retesting the same specimen using a different IgM assay. Antenatal bloods may be retrospectively tested for IgM and IgG reactivity, and avidity. Confirmatory testing in low IgG avidity cases suggestive of recent infection may be via amniocentesis and NAT. Amniocentesis is not completely without risk[35], and is not normally recommended in HIV positive patients[30]. However, it is associated with lower rates of pregnancy loss and has better diagnostic performance versus demonstration of fetal immunoglobulin by cordocentesis (e.g.[36]). In high IgG avidity and/or IgM non-reactive cases suggestive of probable past infection, further investigation depends on gestation and exposure history.

When assessing for suspected congenital toxoplasmosis in a newborn, clinicians should perform a full clinical examination including ophthalmological assessment. Additional considerations include:

- Placental histopathology (e.g. with Wright-Giemsa and immunoperoxidase stains) and NAT[29]. Historical methods include culture (e.g. cell line or mouse inoculation)[30].
- Serology of peripheral blood. Infant IgM and/or IgA reactivity likely suggests congenital infection, although exceptions exist (Table 3). Other indicators include persistent IgG at 6 and 12 months[30,37]. Single avidity scores from newborns may be difficult to interpret without further testing[38,39]. Umbilical cord blood may be contaminated with maternal blood[30].
- Analysis of other sample types including cerebrospinal fluid and urine[37,38]. Methods include NAT and serology (where appropriate). Diagnostic findings include IgM reactivity or target detection on NAT from cerebrospinal fluid[37].
- Imaging for hydrocephalus and calcifications[37].

As for congenital syphilis, despite such considerations, diagnosis of congenital toxoplasmosis can still remain challenging and expert advice should be sought when considering testing, or interpreting test results. Ideally, investigation in women with substantially increased risk of infection should be considered before pregnancy[10], and education regarding preventative measures provided.

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Biography

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Transmission of human cytomegalovirus via breastmilk and potential risks to very preterm infants

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Breastfeeding has clear short-term benefits for the baby[1]. Additionally, based on a prospective long-term cohort study from Brazil, breastfeeding is associated with improved IQ scores and increased educational attainment 30 years later[2]. During lactation, mother-to-infant transmission of viral infections like HIV, hepatitis B (HBV), and human cytomegalovirus (HCMV), may occur. The article presented here will focus on the dynamics of HCMV shedding into breastmilk, describe the short- and long-term risks of HCMV infection of small preterm infants, and options for prevention.

HCMV reactivation during lactation

HCMV, a β-herpesvirus, persists following primary infection for lifetime in hematopoetic CD34+ precursor cells and may be reactivated by stress, transient loss of CD4+ and CD8+ T-cell immunity, IL-6 signalling, cell cycle arrest, and DNA damage[3]. Interestingly, HCMV is also reactivated in healthy immunocompetent seropositive women during lactation[4]. The ratio of HCMV reactivation at any stage of breastfeeding during the first three months after birth is very high (>95%) and nearly equals the maternal seroprevalence[5,6]. The mechanisms leading to viral shedding exclusively into breastmilk are not understood. HCMV seroprevalences in Western Europe, USA, Canada, and Australia range from 40–60%, and are above 90% in South Africa, Brazil, India, Japan, and Turkey[7].

Maternal HCMV reactivation of seropositive mothers during lactation with shedding of viral DNA and virolactia[8] can be detected already in colostrum and normally ends after about three months after birth. According to our experience with individual kinetics of HCMV reactivation in breastmilk of more than 500 healthy HIV-negative breastfeeding mothers of preterm infants, the onset of viral shedding begins with low viral load (<1000 copies/mL) and low infectivity (without detectable infected fibroblast nuclei in short-term microculture) at the end of the first week post partum (p.p.). The onset, the dynamics, and the end of virus shedding into milk is inter-individually different and describes mostly unimodal kinetics (Fig. 1). The viral reactivation during lactation is a strictly self-limited process[8]. Using overnight microculture from cell- and fat-free milk whey, virolactia peaks coincide with viral DNA peak values, varying from 10^3–10^6 copies HCMV DNA/mL of milk whey[9]. The initiation of viral shedding into colostrum shows divergent results. While in Gambia HCMV excretion in colostrum and genital tract were observed in 100% of congenitally infected, and 48% of early infected infants[10], a study from Japan showed, that in seven cases of very low birthweight (VLBW) infants the initial viral load in breastmilk in the first week p.p. ranges between 10 and <1000 copies/mL HCMV DNA[11]. In contrast, an Italian group detected viral DNA in 31 out of 57 (54%) colostrum samples[12].

The HCMV reactivation of HIV-negative mothers during lactation is a local process without detection of a disseminated or compartmentalised infection in plasma, throat or cervical swabs[13–15]. Therefore, HCMV DNA, viral late pp67 transcripts and virions can only be detected in breastmilk cells and cell-free milk whey[8,9,16,17]. Viral cells involved in HCMV reactivation include CD14+ macrophages[9]. However, HCMV-infected milk cells are not essential for virus transmission[8,15]. Several reports found an association between high HCMV viral load in breastmilk and risk of transmission[18,19]. However, other observations seem to be important in the context of prevention[11]. An inverse correlation between milk HCMV-specific IgG avidity and HCMV load was also found[20].

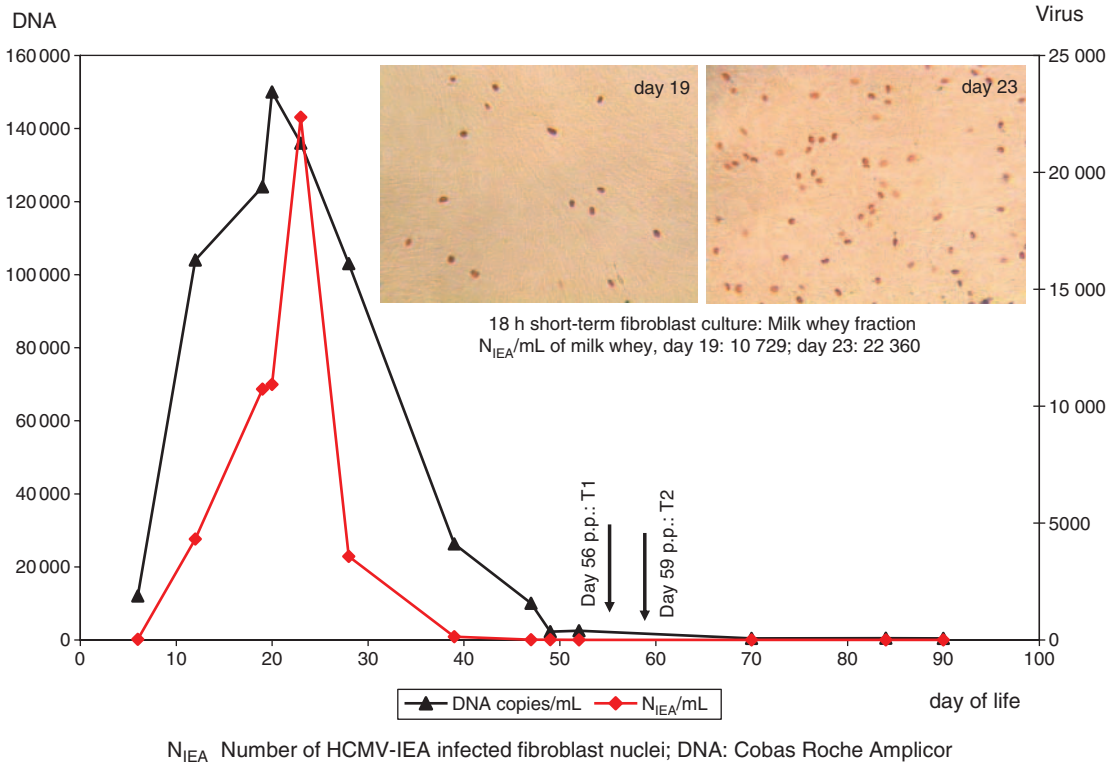

Figure 1. The longitudinal course of human cytomegalovirus (HCMV) reactivation during lactation with shedding of viral DNA and virions from milk whey fraction is shown. The mother delivered healthy preterm HCMV-infected twins (gestational age: 26 weeks). The insert shows infected brownish HCMV-IEA-immunoperoxidase-stained fibroblast nuclei from milk whey at day 19 and 23 after birth. The onset of viral excretion was detected at day 6 post partum (p.p.) with 23 infected fibroblast nuclei/mL, and 12 000 copies HCMV DNA/mL milk whey. Co-culture of total milk cell fraction and fibroblasts stained positive from day 9. At day 6, nested PCR from 64 000 milk cells was negative for HCMV IE1-Ex4 DNA. After peak value of viral DNA and virolactia, milk cells were PCR-negative until day 90 p.p.

In HIV/HCMV coinfected breastfeeding mothers many of the findings in the HIV-negative population are altered. Shedding of HCMV and potentially Epstein–Barr virus (EBV) in breastmilk is associated with HIV-1 transmission by breastfeeding[21]. About 5% of HIV-1-positive breastfeeding mothers had detectable HCMV DNA perinatally in plasma. There was a strong correlation between cervical HCMV DNA detection during pregnancy and later breast milk HCMV levels. Maternal HCMV DNA breastmilk levels and CD4 <450 cells/mm^3 were determinants of HCMV transmission[22]. All HIV-1-infected inocula like genital secretions, breastmilk and blood contain cell-free virus and infected cells[23].

Postnatal HCMV infection of very preterm infants: neonatal entity and prevention

Sepsis-like symptoms (SLS) have been introduced as a term to describe symptomatic postnatal HCMV disease in VLBW preterm infants, comprising apnea and bradycardia, hepatosplenomegaly, grey pallor and distending bowels[24,25]. Following maternal HCMV reactivation and shedding into BM (in 96% of seropositive mothers), the incidence of HCMV transmission to the infant feeding raw, untreated breastmilk was 38% at the age of 3 months corrected age, 18% of the infants had one or more SLS[5]. Virological data could be almost exactly confirmed by a recent report[6] and clinical findings of postnatal CMV infection by many other studies worldwide[26]. The main risks for symptomatic neonatal disease are extremely low birthweight, early transmission, low GA and low infantile IgG titers[27–29]. In a controlled study VLBW preterm infants had a significantly higher incidence of thrombocytopenia, neutropenia and slightly increased C-reactive protein (10–20 mg/L) than matched controls. For the first time in this study clinical parameters could be confined to the entity of postnatal CMV infection in preterm infants (but additional prospective studies with standardized protocols are warranted). All additional parameters included were self-limiting and there was no impact on neonatal outcome parameters like intracranial hemorrhage, periventricular leucomalacia, retinopathy of prematurity and necrotizing enterocolitis (NEC)[30]. However, a large number of cases and case series – including a case from our department which was observed later on – describe severe illness in VLBW infants including pneumonia requiring artificial ventilation, hepatitis and gastrointestinal involvement, with some infants needing antiviral treatment with (val)ganciclovir[11,26,28,31–37].

Options for prevention of virus transmission

Effective prevention of HCMV transmission is only possible through heat treatment of BM. Both long-term (30 min, 63°C) and short-term (5 s, 62°C) pasteurisation methods are effective, but short-term pasteurisation conserves nutritional and immunological relevant

Table 1. Why cryostorage for 10 days is not able to destroy efficiently human cytomegalovirus (HCMV) infectivity in human milk.

Freezing Virolactia DNAlactia See Fig. 1	Control N_{IEA}/mL Copies/mL	18 h –20°C N_{IEA}/mL Copies/mL	4 days –20°C N_{IEA}/mL Copies/mL	10 days –20°C N_{IEA}/mL Copies/mL	30 days –20°C N_{IEA}/mL Copies/mL	60 days –20°C N_{IEA}/mL Copies/mL
Day 19	10 729 124 000	2053 (19.1%) 136 000	n.d.	n.d.	n.d.	n.d.
Day 20	10 926 150 000	n.d.	1966 (18.0%) 123 000	767 (7.0%) 122 000	300 (2.8%) 122 000	13 (0.1%) 117 000
Day 23	22 360 136 000	1001 (4.5%) 241 000	421 (1.9%) 178 000	n.d.	51 (0.2%) 129 000	6 (0.02%) 113 000
Day 28	3568 103 000	1544 (43.3%) 111 000	119 (3.3%) 94 500	96 (2.7%) 92 000	n.d.	0 (0%) 96 000
Day 39	137 26 300	26 (19.0%) 18 200	n.d.	n.d.	n.d.	n.d.
Day 47	7 10 000	1 (14.3%) 10 600	n.d.	n.d.	n.d.	n.d.
Day 49	6 2230	3 (50%) 3620	n.d.	n.d.	n.d.	n.d.

Course of lactation of the HIV-negative mother of preterm twins from Fig. 1. Duration of cryopreservation ranged from 18 h to 2 months at –20°C. Freeze–thawing was performed with aliquots of the raw milk. The results from Fig. 1 correspond to the untreated control. Milk whey was prepared after the given cryostorage time. Using quantitative PCR (Cobas Roche Amplicor) no significant difference related to controls during cryopreservation can be detected. However, short-term 18 h microculture revealed the effect of reduction in infectivity. Residual viral infectivity after cryostorage is given in % of the untreated freshly expressed control milk. Efficiency strongly depends from stage of lactation (see Fig. 1).

components in milk like HCMV-specific antibodies and enzymes[38,39], while Holder pasteurisation does not. Using this method the benefits of BM feeding can be preserved without the disadvantages of CMV transmission.

Freeze–thawing at –20°C for time intervals ranging from 18 h to 10 days is not efficient in viral elimination[38,40,41]. Extended duration of cryopreservation of native breast milk at –20°C from 18 h to 60 days is not efficient for complete virus killing (see Table 1) during decrease of viral DNAlactia and virolactia from peak level to baseline viral shedding, as shown in Fig. 1.

Long-term outcome

An earlier study revealed a significant association of postnatal HCMV infection in infants with birthweight <2000 g and neurologic sequelae and handicaps at 3 years of age[42]. In a more recent study, there was no difference in the neurodevelopmental outcome between VLBW infants with postnatal HCMV infection acquired during their stay in the neonatal intensive care unit and their matched controls at the age of 2–4 years[43]. However, this changed at the age of 4–10 years and 11–16 years: the cognitive outcome of the HCMV infected infants was significantly lower than their controls using K-ABC and HAWIK, the latter with a difference of 93 *v.* 103 ($P < 0.03$)[44–46].

Summary

In conclusion, there is a relevant entity of postnatally acquired symptomatic CMV infection and disease of very preterm infants through raw breastmilk. Actual data are supporting negative influence on long-term cognitive development. Concerning prevention, only heat-inactivation is effective and short-term heat-inactivation preserves the nutritional and immunological capacity of breast milk[39].

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The obstetrician, congenital cytomegalovirus, clinical and diagnostic approaches to the pregnant woman

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There is low awareness of congenital cytomegalovirus (CMV) in Australia. Routine pregnancy serological screening for CMV is not recommended, but all pregnant women should be given advice about CMV prevention. Obstetricians may be asked to see a pregnant woman when serology suggests CMV infection or when features of fetal infection are present on ultrasound. If maternal CMV infection is confirmed, the timing of infection (pre-pregnancy or gestation of pregnancy), must be determined to predict the fetal risks. In addition, it is important to establish whether maternal infection is primary or reactivation. If there is fetal infection, ultrasound can be used to attempt to establish whether the fetus may have been affected. Serial serology, CMV IgG avidity, maternal viraemia (using serum PCR), amniotic fluid CMV PCR, serial fetal ultrasounds, and possibly fetal MRI (magnetic resonance imaging) are investigations that may be useful to predict neonatal outcomes. Timely and accurate counselling is important to optimise maternal and neonatal management.

Primary maternal CMV infection in the first trimester of pregnancy has the greatest risk of adverse fetal and infant outcomes[1]. These include hearing loss, adverse neurodevelopmental outcomes and in severe cases, fetal death[2]. In first trimester CMV infection, approximately 35% of fetuses will develop CMV infection[3,4]. Of these, only 25% will have an adverse outcome due to the infection[4,5]. Thus, it is estimated 10% of women with a primary CMV infection in early pregnancy will have a fetus or infant with an adverse outcome[3]. The rate of fetal infection increases with gestation, but the rate of fetal and infant adverse outcomes decreases with gestation[6]. The rates of adverse outcomes with peri-conception CMV are lower still than in the first trimester[4]. Furthermore, the risks of fetal infection and adverse outcomes are lower with CMV reactivation or re-infection, than with primary infection[7].

Routine serologic CMV screening in pregnancy is not recommended in Australia[8,9]. The potential benefits of routine screening are outweighed by the harms[10]. Screening women at high risk of acquiring CMV infection, ideally pre-pregnancy, may be considered[8]. In addition, CMV serology testing may be indicated in women with signs or symptoms of infection. However, regardless of serological status, pregnant women should be given advice about how CMV is spread and how to reduce exposure to saliva and urine that might contain CMV: this includes advice about handwashing after contact with body fluids, and avoiding sharing food and eating utensils[11].

Most women with CMV infection are asymptomatic[4]. In my practice, I see asymptomatic pregnant women who have had *ad hoc* screening and are CMV IgG positive and/or IgM positive. The differential diagnosis includes pre-pregnancy infection, primary infection (peri-conception or in pregnancy), reactivation/reinfection, or less commonly false positive serology. CMV IgM is a sensitive marker of primary infection, however only 50% of CMV positive individuals have primary infection, as CMV IgM may persist for long periods of time[7,12]. Primary CMV infection can be diagnosed by IgG seroconversion. Antenatal booking serology is kept for 1 year. CMV IgG avidity may be useful to determine timing of CMV infection, especially if no other sample serology is available for comparison[7]. High avidity during the first trimester excludes primary infection within the preceding 3–4 months[7]. Low avidity suggests infection within the previous 3 months. Change in avidity may also be used to predict timing of infection[13]. Maternal CMV viraemia using polymerase chain reaction (PCR) may be also useful to establish the time of infection[14].

Once the diagnosis of primary maternal CMV in pregnancy, or periconception is established, determining fetal infection will assist in predicting infant outcomes. Amniotic fluid CMV PCR is the most

accurate method of detection of fetal CMV infection[15]. Ideally an amniocentesis should be performed after 20–22 weeks gestation, and a minimum of 6 weeks after the primary infection[16]. A negative result before then may be falsely reassuring, and a repeat amniocentesis with a small risk of miscarriage (0.5%), may be required. If the amniotic fluid CMV PCR is negative, the risk of the fetus being infected at birth is small (8%), and the risk of an abnormal outcome is low (~0.5%)[3].

Fetal ultrasound can be used to predict adverse fetal/ neonatal outcomes of fetal CMV infection. The ultrasound features of congenital CMV are non-specific, but include microcephaly, echogenic bowel, intrauterine growth restriction, hydrops fetalis, cerebral ventriculomegaly, brain calcifications and an enlarged placenta[5,17,18]. The risk of adverse fetal/ neonatal outcomes when there are ultrasound abnormalities and proven fetal infection is ~46%[3]. This risk is significantly reduced to 13%, when there are no ultrasound abnormalities[3]. However, if there are no ultrasound abnormalities, ongoing ultrasound surveillance is recommended, as ultrasound evidence of CMV damage may develop later[15]. MRI may give additional information in cases of confirmed fetal infection, especially if the fetal ultrasound shows no abnormality[5].

Women who have possible or proven CMV infection in pregnancy should be counselled by experienced clinicians[10,19]. The role of maternal therapy to prevent adverse fetal and neonatal effects of CMV infection is uncertain[20]. Ongoing research into the role of CMV hyperimmune globulin is in progress[21]. Termination of pregnancy may be an option for some women, particularly if there is fetal infection and/or ultrasound evidence of fetal sequelae. The availability and timing of termination of pregnancy varies, as laws differ between states and territories in Australia[22]. A previous study has found that 17% of women with a CMV diagnosis in the first trimester will terminate the pregnancy, before undergoing an amniocentesis[22]. If fetal or maternal CMV in pregnancy has been diagnosed and the pregnancy continues, neonatal investigation is recommended. Clinical examination and neonatal saliva PCR are the initial methods of choice[23], and placental examination may be useful. When neonatal CMV infection is detected, neonatal surveillance for hearing loss and long-term outcomes is recommended to reduce the risk of adverse sequelae[24]. The role of antiviral therapies is less certain[20].

It is well recognised that CMV infection may persist[25]. The optimal time to avoid pregnancy after CMV infection is not known. However postponing pregnancy for a period of 6–12 months may be advisable after primary CMV infection, as periconception infection is also associated with fetal effects[4,26].

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Biography

Dr Antonia Shand MBChB, FRANZCOG, DDU, CMFM, M. Med (Clin. Epi.) is a Maternal-Fetal Medicine subspecialist at the Royal Hospital for Women in Sydney. She went to medical school in Otago, New Zealand and completed her obstetric and maternal fetal medicine subspecialty training in Sydney and in Perth. She also works in the Clinical Population and Perinatal Health research group at the Kolling Institute of medical research at the University of Sydney. Her clinical and research interests include infection in pregnancy as well as complicated pregnancies.

Animal models of human cytomegalovirus congenital infection

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Human cytomegalovirus (HCMV) infection is highly species-specific, which means that it is unable to productively infect laboratory animals. Despite this caveat, studies of animal CMV counterparts in their natural hosts have revealed significant correlations with observed neuropathological effects of congenital HCMV infection and have improved our understanding of host responses to vaccination. The biological relatedness between human and animal CMVs has been confirmed by phylogenetic analyses; the conservation of 'core' genes that are essential for virus replication as well as genes that contribute similar mechanisms for virus persistence in their respective host species. The common animal models of HCMV congenital infection include Rhesus CMV (RhCMV), guinea-pig CMV (GPCMV) and mouse CMV (MCMV). Whilst animal models of CMV do not fully recapitulate HCMV infection, they each offer specific advantages in understanding HCMV congenital/perinatal infection (summarised in Table 1).

Transplacental transmission and neonatal infections

The placentae of the guinea-pig and the rhesus macaque are structurally similar to the human placenta[1]. Experimental infections with RhCMV and GPCMV result in foetal infection, with clinical manifestations that include CNS involvement and (for GPCMV) sensorineural hearing loss (SNHL)[2,6,7]. Systemic maternal infection causes syndromes deleterious for the developing foetus, (e.g. intrauterine growth restriction) with the incidence of foetal morbidity and mortality being highest when transmission occurs during early gestation[2,3]. These key pathological features are similar to congenital HCMV infection[15].

Despite poor transplacental transmission of MCMV in the laboratory setting, direct injection into the foetus or the newborn pup has been shown to mimic HCMV-induced congenital disease[12,13]. Similar to RhCMV, the susceptibility of neuronal stem cells to MCMV infection is maturation stage-dependent, with a rapid resistance to infection of the brain developing after birth[16]. MCMV also infects the auditory nerve spiral ganglion and cochlea of newborn pups with measurable cytopathic effects and neuronal loss, and thus offers an amenable model for studying viral and host factors that contribute to SNHL[17].

Evaluation of antiviral therapies to ameliorate effects of congenital infection

Current antiviral therapies for HCMV target the viral replicative machinery (e.g. ganciclovir, valganciclovir, foscarnet and cidifivor)[18,19]. However, due to their toxicity, none are licenced for use during pregnancy and only ganciclovir/valganciclovir (that target the

Table 1. Animal CMV infections: comparisons of their features and potential for use as a model for HCMV congenital infection.

Animal	CMV	Viral genome	Model strengths	Model challenges
Rhesus Macaque	RhCMV	221 kb	Anatomically relevant[1]	Very expensive and long gestation[2]
			Similar foetal CNS disease Cochlear infection[2,3]	Low availability of immunological reagents
			Drug pharmacokinetics/toxicity[2,4]	Few RhCMV-naive colonies[2]
			Vaccine immunogenicity[5]	
Guinea-pig	GPCMV	233 kb	Placental and foetal infection[6]	Low availability of immunological reagents
			Similar foetal CNS disease Cochlear infection; IUGR[6]	Cost is high for vaccine and drug studies
			Similar SNHL sequelae[7]	
			Proven model for drug efficacy[6,8,9]	
			Proven model for vaccine immunogenicity and efficacy[5,10,11]	
			Highly refined model with respect to virus dose, timing of infection, end organ disease[9]	
Mouse	MCMV	230 kb	Good newborn infection model[12,13]	Poor transplacental transmission[6]
			Excellent knowledge of virus-host interactions[14]	Not suited for vaccine/drug efficacy studies targeted at preventing congenital infection
			Availability of immunological reagents and mice with targeted immune defects	
			Relatively inexpensive and short gestation allows for high-throughput antiviral/immunogenicity studies	

HCMV-encoded UL97 kinase) are administered to symptomatic HCMV-infected newborns at high risk of SNHL[18,20,21]. Despite similarities in the pharmacokinetics between humans and rhesus macaques, and similar sensitivities to HCMV antivirals, assessments of drug efficacies have not been performed due to the expense of this model[2,4]. By comparison, GPCMV model has proved a highly valuable model to evaluate the action of hexadecyloxypropyl-cidofovir (brincidofovir or CMX001) in the reduction of foetal morbidity, virus load and the manifestation of SNHL[8]. Although GPCMV is resistant to ganciclovir, the generation of GPCMV/HCMV UL97 chimaeric viruses will enable future antiviral testing of ganciclovir *in vivo*[9]. Poor placental transmission by MCMV precludes the evaluation of antivirals on foetal infection. Nevertheless, the newborn infection model offers excellent prospects for rapid screening of novel drugs on CNS infection and disease, including SNHL. Maribavir, an inhibitor of the UL97-kinase, has become a promising alternative to ganciclovir due to its reduced toxicity: its efficacy in ameliorating SNHL in newborns awaits testing in animal model systems[19].

Vaccination studies

Because maternal immunity reduces the severity of congenital CMV disease, the development of a vaccine is a high priority[22]. The GPCMV model has been used extensively for evaluating vaccine efficacy, by virtue of the ability to quantify the maternal immune responses, virus loads, as well as developmental sequelae[10]. Several GPCMV vaccines (live attenuated, subunit and DNA) administered before conception, have been evaluated[5,11]. Whilst sterilising immunity to any vaccination program has not been demonstrated,

the model has informed HCMV vaccination strategies with respect to choice of the immunogen and adjuvant as well as identifying diagnostic correlates of foetal protection, such as the magnitude of the maternal neutralising antibody response to vaccination and reduction in maternal viraemia[5,6,11,23]. The use of vectored approaches for the delivery of subunit GPCMV vaccines that provide both cellular and humoral immunity also significantly reduce the incidence of congenital GPCMV infection[5,24]. Notably, transplacental transmission of GPCMV has been observed in dams that had been previously infected, a feature in common with the evidence of symptomatic HCMV congenital infections resulting from maternal re-infections during pregnancy[25].

To date, there have been few RhCMV vaccination studies, confined to immunogenicity, rather than efficacy studies. This is due in part by the paucity of seronegative colonies. Nevertheless, the RhCMV model has been useful to identify optimal vaccination regimes and immunogens that elicit strong cellular and humoral immune responses, using heterologous DNA prime-protein boost approaches[5]. Notably, recent studies of RhCMV have uncovered novel, diverse and highly promiscuous CD8+ T-cell repertoires from macaques immunised with a live RhCMV vaccine deleted of HCMV counterparts responsible for cell tropism. The results have implications for the use of HCMV deletion mutants in directing the CD8+ T-cell repertoire and for their use as a vector for delivery of other immunogens[26].

The MCMV model has been instrumental in our understanding of mechanisms of innate and adaptive mechanisms of host resistance to infection[14]. The availability of immunological reagents has facilitated the characterisation of both humoral and cell-mediated responses to MCMV using live attenuated, subunit, DNA and vectored vaccines[27,28]. Although poor transplacental transmission precludes laboratory studies of vaccine efficacy, there is potential for the MCMV model to measure the potency of maternal immunity from vaccinated dams in restricting perinatal infection of newborn pups.

Other animal models

The potentials of rat CMV (RCMV) or porcine CMV (PCMV) as models of HCMV congenital infection have not been explored. PCMV is of interest because natural maternal infection results in transplacental transmission and thus it may provide an authentic evaluation of vaccination efficacy[29]. There has been a recent report of placental infection of rats with a novel RCMV[30]. If further studies demonstrate foetal infection, then this model would provide a highly amenable approach to testing intervention strategies.

Future Perspectives

Several advances are facilitating refinement of the above animal models for congenital HCMV:

- The implementation of chimaeric CMVs that express authentic HCMV immune/drug targets and 'humanised' animal models that dissect protective immune responses[9,31];
- The exploitation of viral 'immune evasion' proteins either as targets for the immune response or their deletion in live attenuated viral vaccines[32,33];
- The implementation of live imaging technologies to track virus dissemination *in vivo*[9];
- The identification of viral and cellular determinants that dictate HCMV species-specificity may allow future cross-species studies of HCMV infection[5].

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Biography

Helen Farrell completed her PhD at the University of Western Australia under the mentorship of Geoff Shellam in 1989, exploring virus-host relationships using the mouse CMV model. She has continued to identify and characterise herpesvirus determinants of pathogenesis and persistence during her postdoctoral career in the UK and in Australia. She is currently a Senior Research Officer at the School of Chemistry and Molecular Biology and the Centre for Children's Health Research at the University of Queensland.

Obituary: Professor Geoffrey Randolph Shellam

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It is a privilege for all of us to present a brief tribute for the life of Geoffrey Shellam, an admired colleague and friend, to some for over 50 years. Geoff's scientific intuition, his integrity and his scientific leadership were second to none.

The PhD years

Geoff was Gus Nossal's first PhD student at the Walter and Eliza Hall Institute in 1966, and in 1967 was joined by Richard Stanley who studied colony stimulating factors under the late Donald Metcalf. A fateful conjuncture, as Geoff introduced Richard to Pamela Featherstonehaugh, who became Richard's wife; and Richard introduced Geoff to his sister Fiona, whom Geoff in turn married. During his PhD, Geoff studied immunological tolerance. One particularly intriguing experiment involved injecting newborn rats repeatedly with minuscule doses of bacterial flagellin. This resulted in tolerance as measured by the formation of immobilising antibodies, but only if the injections were given daily (including Sundays) over several weeks. The group called this ultra low-zone tolerance. Geoff had been at the Walter and Eliza Hall Institute as a CSL cadet and on graduating went to a research post at CSL. However he left and travelled to London to work with Avrion Mitchison on tolerance, did very well and stayed for 5 years.

The UWA years

Geoff won the prestigious Eleanor Roosevelt International Cancer Fellowship to research at the National Cancer Institute in Maryland, USA before coming to The University of Western Australia as a Postdoctoral Fellow in 1977. Geoff's work in Perth was supported by the NHMRC, and in an increasingly competitive environment, he was successful in gaining peer-reviewed NHMRC research support for the rest of his life. In 1985, he succeeded Neville Stanley in the Chair, which he held with great distinction for 30 years. Geoff was a great addition to UWA Microbiology, with enormous calibre, expertise and deep interest in NK cells. He initially continued his work on cell-mediated cytotoxicity in the rat lymphoma model, and established a bright, keen research group with excellent postgraduate students. However, he quickly recognised the potential of the murine CMV system initiated by Jane Grundy in her PhD studies and realised it provided an ideal model in which to further investigate the role of NK cells and host genetic resistance/susceptibility.

Geoff's research group was able to utilise similar techniques and concepts to study flaviviruses, successfully investigating the *Flv* gene associated with flavivirus resistance in the mouse, and eventually mapping the *Flv* gene to a single chromosome 5 locus. He also employed murine CMV as a vector for developing an immune-contraceptive vaccine to control wild mouse plagues, and using his experience with host genetic resistance mechanisms, was able to overcome the host innate resistance that occurred. Geoff's imagination and sense of adventure led him to use some of this knowledge to tackle mouse plagues in the wheat fields with a recombinant murine CMV that induced autoimmune destruction of ovarian follicles. Geoff had a strong interest in fieldwork associated with some of the more inhospitable parts of the world. In the Kerguelan and Macquarie Islands, he collected cloacal swabs and blood from penguins for virus isolation, and wild mouse populations for his MCMV studies into genetic resistance patterns. This final passion researching a devastating viral disease (infectious bursal disease) in penguin chicks necessitated several visits to Macquarie Island, an adventure typical of him.

Geoff with these colleagues and protégés including Jane Allan, Greg Bancroft, Mariapia del Esposito, Helen Farrell, Jane Grundy, Patricia Price, Alec Redwood, Tony Scalzo, and Lee Smith used mouse genetics to unravel key aspects of innate immunity to murine CMV as a model for the human viral disease, and with John Mackenzie, Mark Sangster and Nadia Urosevic in their work on flaviviruses. One of their greatest triumphs was defining the role of NK cells in protection again murine CMV infection and then with Tony Scalzo, mapping a key gene (*Cmv1*) that encoded this resistance. They showed this had a dominant effect and was mapped to the distal region of mouse chromosome 6. The effect of *Cmv1* was subsequently shown to be mediated via NK cell control of viral infection and the locus was mapped to the NK cell gene complex (NKC), a region which encodes both inhibitory and activation NK cell

receptors. In later years the work with Tim Booth, Megan Lloyd, Alec Redwood and Lee Smith examined how murine CMV developed strain variation including in wild type strains, and how these strains influenced immune evasive genes provided key models for other herpesviruses.

A lifelong legacy

Geoff was an original and dedicated teacher at both undergraduate and postgraduate levels. His teaching involved all aspects of infectious diseases, including virology, microbiology, immunology, molecular biology and public health. A Master of Science course that he introduced became unexpectedly popular and successful. Geoff's gifts as an administrator involved not only his superb department, but also being Co-Director of the Marshall Centre for Infectious Diseases Research and Training. He was a model corporate citizen joining a wealth of national and international scientific committees, peer group reviewing bodies and editorial boards. He was always willing to serve, as head of department for three decades, as elected President of the Australasian Society for Immunology of which he was made an honorary life member in 2012, as founding co-director of the Marshall Centre at UWA and on the scientific advisory committee of the Australian Centre for HIV and Hepatitis Virology Research.

The first impression of Geoff remains for many of us, a man with a formidable intellect, a gentleman, an irreverent sense of humour and a great sense of fun and adventure. A mentor. Geoff's work was an inspiration. He was able to deliver precision to a very descriptive field complicated by the large size and number of genes in 'his' herpesvirus, murine cytomegalovirus, as well as the complexity of the immune response, which was relatively poorly defined at the time.

The seminal studies from Geoff's research group have provided the basis for dissecting and delineating so many areas of virus-host interaction, including the contribution of host resistance genes to infection outcome. Along with his excellent group, Geoff's work dissected the various interplays between innate and adaptive host immune responses, the contribution of virus immune evasion genes, and genetic variation in these genes, to the establishment of viral persistence.

Vale, Geoffrey Shellam and rest in peace. We will miss your great good humour and favourite salutation 'old boy'. It has been a pleasure and an honour for many of us to know you. Those of us fortunate to have known you, and those who continue to benefit from your highly intelligent research insights, thank you for the good you have done for Australia and the world.

Conference report: 19th ISHAM Congress, Melbourne

Prof. Wieland Meyer

Chair 19th ISHAM Congress and General Secretary ISHAM

The 19th Congress of the International Society for Human and Animal Mycology (ISHAM), in-conjunction with the 2015 Mycology Masterclass, was proudly organised by the Australian New Zealand Mycology Interest Group (ANZMIG), a special interest group of the Australasian Society of Infectious Diseases (ASID). It took place from the 4–8 May 2015 at the Melbourne Convention and Exhibition Center.

The Congress was organised under the leadership of Prof. Wieland Meyer, who chaired the local organising committee, including Dr Sarah Kidd, A/Prof. Sharon Chen, Prof. Monica Slavin, Sue Coloe, A/Prof. Debbie Marriott, Dr Orla Morrissey, Dr Tom Gottlieb, Prof. Tania Sorrell, A/Prof. Mark Krockenberger, Prof. David Looke and E/Prof. David Ellis. The accompanying Young ISHAM meeting was organised by Dr Michaela Lackner, Cecilia Li, Nenad Macesic. Special thanks go to the staff of The Meeting People our PCO under the leadership of Lesley Woods.

The 19th ISHAM Congress was generously sponsored by Astellas, Merck Sharpe & Dhome, Pfizer, Gilead, Elsevier, Marie Bashir Institute, Oxford University Press, Melbourne Convention & Visitors Bureau, Majestic Opals, Cape Cod Associates, and Mayne Pharma.

The meeting attracted 667 delegates from 48 countries, with 33.7% coming from Australia and 66.3% from overseas. It was preceded by two days of workshops, on 2–3 May 2015, and followed by a one-day workshop on 9 May 2015, taking place at RMIT and the Alfred Hospital, on topics from MALDI-TOF, Histopathology, Therapeutic Drug Monitoring, Antifungal Susceptibility Testing, Infections in the Immunocompromised Host to BioloMICS. The Young ISHAM day on 3 May 2015 featured three educational sessions, covering topics from 'Where to publish' and 'How to review a scientific paper' to 'Mycological entrepreneurship'. It also gave 24 young researchers the opportunity to present their scientific findings as short talks and 88 as YISHAM posters.

The formal meeting was opened by the Lord Mayor of Melbourne Robert Doyle and the ASID President Prof. Cheryl Jones. The opening plenary given by Professor Sarah Gurr, Chair in Food Security at Exeter University, entitled: 'Mycopia: Fungal Allies and Adversaries', seamlessly linked environmental, food safety, agricultural and medical mycology. The eight plenary lectures covered themes reaching from 'New Insights in Candidaemia' (Prof. Tania C. Sorrell, AU), 'Insights in fungal pathogenicity drawn from proteomics studies' (Prof. Marilene Henning-Vainstein, Brazil), 'Modelling of human antifungal dosages' (Prof. William W. Hope, UK), 'Animal to human transmission new threat of Sporotrichosis' (Dr Isabella Dib F. Gremião, Brazil), 'Antifungal resistance – threats, trends and targets' (Prof. Richard Cannon, New Zealand), 'How can animal models inform clinical practice?' (Prof. John Perfect, USA), 'Advances in Mucormycosis' (Prof. Olivier Lortholary, France) to 'Virulence mechanisms shared by fungi that infect humans, animals and plants' (Prof. Joseph Heitman, USA). The main program had many scientific highlights within the 36 symposia, featuring 323 oral presentations, representing the four themes within the program: clinical, basic science, translational and one health aspects of medical mycology. 488 posters were presented at the Congress, showcasing high quality original research and state of the art reviews. There were three industrial symposia organised by Pfizer, MSD and Astellas. The Congress also featured 18 ISHAM working groups, allowing them to present their activities to the general scientific community.

In conjunction with the 19th ISHAM Congress a special issue of Microbiology Australia on Medical and Veterinary Mycology was published in May 2015 by the Mycology Interest Group of ASM, under the guest editor ship of Prof. Wieland Meyer featuring one editorial, three In Focus and 13 Under The Microscope articles, highlighting to the global scientific community the broad range of Medical Mycology activities in Australia.

The Congress offered plenty of opportunities to network either during the numerous poster sessions or the YISHAM party at Gasolina, the welcome reception, which took place at the Melbourne Convention Center and the Congress dinner at the amazing Myer Mural Hall.

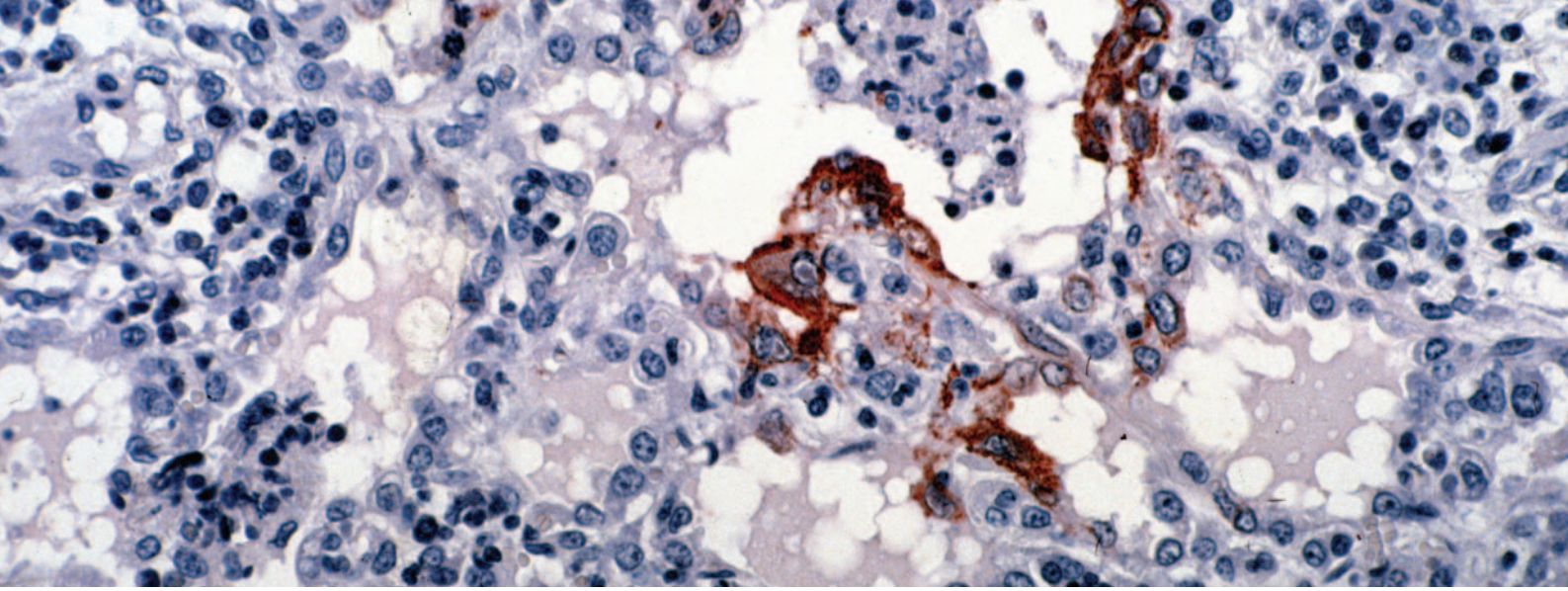
The 19th ISHAM Congress was also the official meeting place of the members of ISHAM, which held its general assembly on 8 May 2015. During the General assembly the new ISHAM council was elected: Prof. Malcolm Richards (UK) – President, Prof. Wieland Meyer (AU) - General Secretary, Dr Donna MacCallum (UK) – Treasurer, Prof. Arunaloke Chakrabarti (India) - President Elect, Dr Ruth Ashbee (UK), Prof. Yee Chun Chen (Taiwan), Prof. Jacques Meis (The Netherlands) and Prof. Ryoji Tsuboi (Japan) – Vice Presidents, Dr Marcus Teixeira, Brazil/USA – Vice President YISHAM and ex officio Dr Oliver Kurzai (Germany) – Chief Editor Medical Mycology Case Reports, Dr Karl Clemens (USA) – Chief Editor Medical Mycology and Prof. Neil Gow (UK) – Past president.

The society awarded its highest award, the Lucile George Medal to Prof. John Perfect (USA) for his outstanding contribution to clinical mycology and Prof. Luigina Romani (Italy) for her outstanding contribution to basic mycological science. Honorary ISHAM membership was awarded to Prof. Bodo Wanke (Brazil) for his outstanding contribution to human and animal mycology, and the distinguished service awards were awarded to Prof. Ira Salkin (USA), Chief Editor of the society journal Medical Mycology, Prof. Aristea Velegraki (Greece) and Prof. Sybren de Hoog (The Netherlands) for their outstanding contribution to the Society.

It also elected the next host country of the 20th ISHAM Congress, which will take place in Amsterdam, The Netherlands in 2018.

Overall the 19th ISHAM congress was a great success featuring all aspects of human and veterinary mycology.

Some members of the organising committee: Cecilia Li, Sarah Kidd, Orla Morrissey, Debbie Marriott, Wieland Meyer and Sue Coloe.



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Confirmed Plenary speakers

Professor Peter Hawkey
University of Birmingham
Nosocomial infection control and antibiotic resistance

Professor Dan Andersson
Upsalla University
Environmental pollution by antibiotics and its role in the evolution of resistance

Assoc Prof Susan Lynch
University of California San Francisco
Colitis, Crohn's Disease and Microbiome Research

Dr Brian Conlon
Northeastern University, Boston
Drug discovery in soil bacteria

Professor Anna Durbin
Johns Hopkins
Dengue and vaccines

As with previous years, ASM 2016 will be co-run with EduCon 2016: Microbiology Educators' Conference

Watch this space for more details on the scientific and social program, speakers, ASM Public Lecture, workshops, ASM awards, student events, travel awards, abstract deadlines and much more..

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