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# Pathogenesis of congenital cytomegalovirus infection of the central nervous system

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## Abstract

*Human Cytomegalovirus (HCMV) is the leading viral cause of congenital infections in the central nervous system (CNS). HCMV infection in the brain is accompanied with wide spread encephalitis and developmental abnormalities of newborn brain which may result in severe long term sequelae. Due to species specificity of CMVs, animal models are frequently used for HCMV pathogenesis research. Murine cytomegalovirus (MCMV) shares many biological similarities to HCMV and therefore mouse model is most frequently used to study the pathogenesis of congenital HCMV infection. MCMV establishes productive infection in the brain parenchyma of newborn mice which leads to extensive non-necrotizing multifocal wide-spread encephalitis characterized with infiltration of both components of innate and adaptive immunity. As a result, impairments in postnatal development of mouse cerebellum lead to long term motor and sensor disabilities. Extrapolated data from murine model indicate that CMV infection and inflammation in the developing CNS alter normal tissue programs in developing brain and, thus, are responsible for the neurological disorders associated with congenital CMV infection. High rate of sequelae following congenital CMV infection and insufficient antiviral therapy in perinatal period assigned CMV-specific vaccine as the highest priority of modern medicine.*

## CMV INFECTION: PUBLIC HEALTH ISSUE

Cytomegalovirus (CMV) is a large DNA virus which belongs to a family of  $\beta$  herpesviridae. Human cytomegalovirus (HCMV) is ubiquitous pathogen in humans, infecting over 50% of the world population (1). Like all herpes viruses, human CMV (HCMV) establishes life-long latency in infected host and can be reactivated depending on the host immune status. In immunocompromised patients and transplant recipients HCMV acts as a devastating opportunistic agent inducing life treating pneumonitis or encephalitis. Public health importance of HCMV infection is especially highlighted in newborns and prematures: HCMV is the leading viral cause of congenital infections resulting in long term neurological defects (2, 3). Intrauterine HCMV infection usually occurs during primary maternal infection when the rate of mother to fetus virus transmission is over 40% (4) with the 20% to 25% risk of postnatal development of neurological impairments in infected infants (5). Although most of the infected newborns have asymptomatic disease, the majority of children who suffer from symptomatic HCMV infection and, even more importantly, 10–15% of children with asymptomatic HCMV disease will develop severe neurologi-

cal impairments including deafness, mental and psychomotor retardation, blindness, microcephaly, hydrocephalus, and cerebral calcifications (6–9). This recognition of the clinical importance of invasive HCMV disease in the setting of immunodeficiency and in children with congenital HCMV has induced the development of a HCMV vaccine as top priority for the 21<sup>st</sup> century by the US Institute of Medicine (10).

## **PATHOGENESIS OF CMV INFECTION IN DEVELOPING BRAIN**

Congenital HCMV infection is a result of virus transmission from infected mother to child in three possible ways: intrauterine (transplacental), intrapartum (exposure to virus in the genital tract) and post-natal (acquisition via breast milk). Systemic HCMV infection affects majority of organ systems from which reticuloendothelial and nervous system are most heavily affected. Symptoms of acute HCMV infection in newborns include jaundice, hepatosplenomegaly, thrombocytopenia and microcephaly. While affection of reticuloendothelial system does not result in long term disabilities, infection in developing central nervous system (CNS) causes significant morbidity.

The exact route of CMV infection to developing CNS is still insufficiently defined. Several mechanisms of virus entry into the brain parenchyma have been proposed for different neurotropic viruses, including herpes viruses, based on the data extrapolated from both *in vitro* experiments and the ones performed on animal models. Following infection of an infant CMV establishes viremia and colonizes different organs, including the brain. Mechanisms of virus entry into the CNS are still insufficiently defined and several routes were proposed: infection of endothelial cells forming the blood-brain barrier (BBB) and viral spread to astrocyte processes (11, 12); virus spread through cerebrospinal liquor and infection of epithelial cells of the chorioid plexus (13); and infection via infiltration of monocytes. In early postnatal period monocytes populate the brain to become microglia cells (14). Since CMV replicates in monocytes (15), these cells could serve as carrier of the virus entry into developing CNS. Moreover, CMV infection of endothelial cells induces monocyte extravasations and infection which facilitates virus propagation into developing brain (16). For different viruses, including CMV, the capability to infect endothelial cells, the main structural elements of BBB, pronouncedly facilitates the route for entering brain parenchyma (17–20). Altered expression of tight junctions, resulting in disintegration of the BBB, is observed following viral infection of endothelial cells in various viral infections (21–23), but data regarding BBB disruption during congenital CMV infection are rare. Studies performed by using Evans blue dye did not confirm the disruption of BBB following CMV infection of newborn mice (24). Some explanation may be that BBB in early postnatal (PN) period is still not completely mature, which is evident by decreased distribution of glucose transporter (GLUT-1) in mice, one of the first BBB

markers on brain endothelial cells in the early postnatal period (25). Minimum level of GLUT-1 expression is observed at day 7 PN and reaches the level typical for mature BBB at day 14 PN. Other interendothelial junction-associated proteins: zonula occludens protein (ZO-1), occludin and  $\beta$ -catenin also accomplish their adult extent at day 14 PN (25). Additional pathway of CMV entry into developing CNS is the infection of ependymal cells of the chorioid plexus which leads to virus dissemination into the cerebrospinal fluid (CSF) and consequent infection of brain parenchymal cells (13).

In a murine model of congenital HCMV infection peripheral inoculation of the virus into newborn mice results in systemic viremia characterized by both cell associated and cell-free virus present in the blood (26). Therefore virus CNS colonization by CMV after early postnatal infection is mediated by both direct viral infection of cells in contact to free virus and infiltration of infected cells that actively populate developing brain.

Various aspects of the pathogenesis of CMV infection in developing CNS are investigated by studying the cell tropism, infectious dynamics of CMV infection and the effects of CMV infection on proliferation, regeneration and differentiation of neural cells. Both *in vitro* and *in vivo* studies showed that human and animal neural progenitor cells are fully permissive for CMV infection (27, 28). Studies performed on cultured human brain cells showed that neurons present the site of persistent infection, while lytic infection occurs in glial cells (29). When mature, neurons lose the susceptibility to CMV infection; in contrast to differentiated glial cells where viral replication proceeds (30). Astrocytes, cells that constitute majority of brain parenchyma, support virus replication (31) while in microglia cells viral antigens can be detected, but productive CMV infection cannot be determined (32).

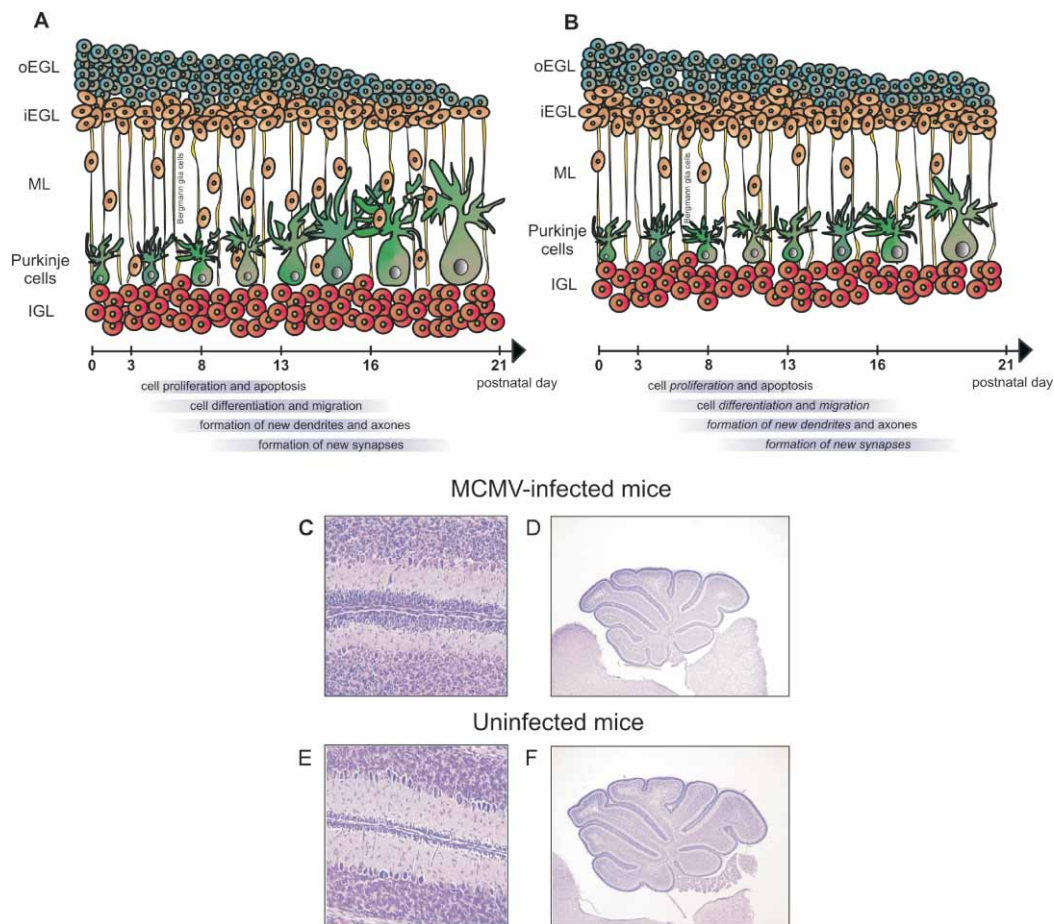
HCMV in developing CNS induces focal, but widespread non-necrotizing encephalitis (33, 34), characterized with typical pathohistological findings – foci of infected cells coupled with inflammatory response (33). Typical pathohistological lesions – glial nodules that surround infected neurons frequently coupled with neurophagy, perivascular cuffing around endothelial cells, mononuclear cell infiltration in both brain parenchyma and meninges as well as periventricular necrosis are readily described in autopsied cases (35). Similarly, mouse cytomegalovirus has shown the same pattern of pathological findings in infected brain (24). Lytic infection of both neurons and glial cells in mouse brain terminates within three weeks post infection but pathohistological lesions reside in the CNS for several months afterwards (26). This argues for either virus persistence in the brain that constantly primes immune response or consequent development of immunopathology in this immunologically privileged organ (24).

### MECHANISMS OF NEURONAL IMPAIRMENT IN CMV INFECTED NEWBORN BRAIN

Imaging studies suggested greatly for HCMV infection involvement in neuronal impairment during early development of the CNS (36–38). Congenital brain infection can result in variety of neurological disorders ranging from severe structural damage of the brain with profound cognitive delays to disorders of perceptual senses such as hearing or visual loss. Most common finding in autopsied infected infants is cerebellar hypoplasia (7), while others include periventricular calcifications, ventriculomegaly, delayed myelination, periventricular occipital cysts, lysencephaly, hippocampal dysplasia, white matter gliosis and cortical neuron migration disorder (39, 40). White matter lesions coupled with anterior temporal lobe cysts on MR images are suggestive for CMV infection (41). HCMV is thought to be the most com-

mon cause of acquired hearing loss in the US with up to 15% congenitally infected children exhibiting hearing loss (42–45). Interestingly, retinitis associated with congenital HCMV infection can recur later in life, suggesting that retina could be the site of viral persistence in these patients.

Mechanisms of developing brain injury caused by CMV are insufficiently understood, mostly due to the limited number of autopsy cases with a comprehensive description of the pathological changes detected in infected infants. Virus-induced vasculitis might be responsible for loss of vascular supply to regions of the developing brain, resulting in maldevelopment (35). Other investigators have postulated direct cytopathic effect of CMV on developing neurons and glial cells (46) resulting in impaired neuronal migration and cellular positioning during postnatal brain development (7).



**Figure 1.** Postnatal cerebellum development in newborn mice is impaired during perinatal MCMV infection. (A) During postnatal cerebellum development in newborn mice granule cells in the outer part of the external granular layer (oEGL) proliferate and move into inner part of the EGL (iEGL) where they differentiate in order to migrate into internal granular layer (IGL) of the cerebellar cortex. Proliferation and differentiation of granule neurons is controlled by number of genes that are expressed either by Purkinje cells or granule cells itself. In parallel to proliferation of granule neurons, Purkinje neurons arborize their dendrites in the molecular layer of the cerebellar cortex and form synapses with other neurons. (B) In MCMV-infected newborn mice impaired proliferation and delayed migration of granule neurons from iEGL into IGL is observed, as well as impaired morphology of Purkinje cells. As a result (C) increased thickness of EGL and (D) decreased cerebellar area are observed, as compared to (E, F) uninfected control mice. All figures present brains from 9 days old newborn mice. Cresyl violet staining. Magnifications 4x (D, F) and 10x (C, E). For details of experimental procedure see the reference (24, 58).

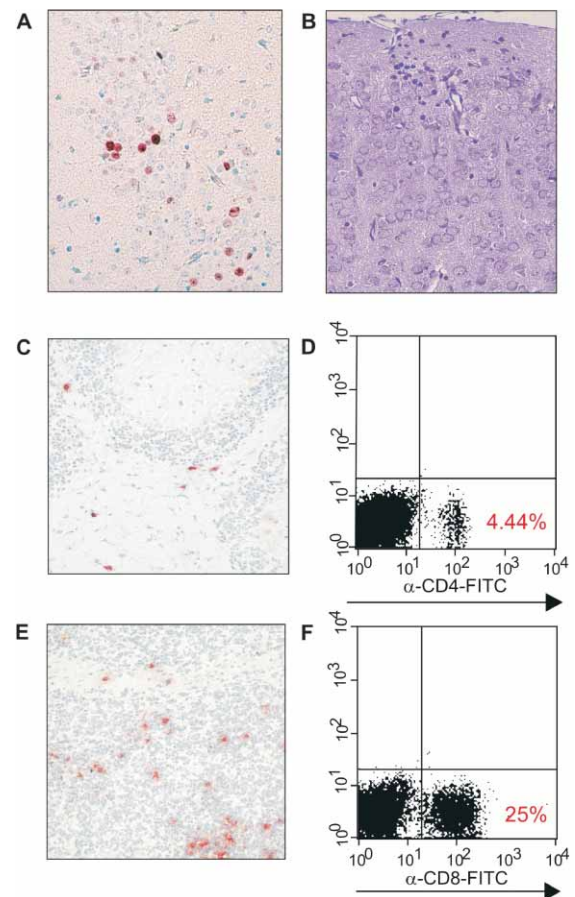
Some evidence point out that HCMV replicates in the placenta and can cause its inflammation and dysfunction (47). This can indicate that congenital CMV disease is in part a syndrome of placental insufficiency and that sequelae are developed as a result of systemic blood insufficiency to the fetus (48).

The investigation of *in vivo* pathogenesis and immunology of HCMV infection in the CNS has been limited by the strict species specificity of the virus. Murine CMV (MCMV) shows significant homology in genomes, exhibits conserved tissue tropism and temporal regulation of gene expression and display similar pathogenesis to HCMV infection (49), which allows the use of MCMV as a model of human infection [50]. In the early postnatal period (days 1–21) mouse cerebellum undergoes significant morphological and developmental perturbances in which granule neurons in the external granular layer (EGL) proliferate and differentiate in order to migrate into deeper parts of the cerebellar cortex, mainly into internal granular layer (IGL) (Figure 1A). This process is strictly controlled by number of genes which are either intrinsically expressed in granule neurons, or have extrinsic effect on granule cell proliferation and differentiation. These genes are mainly expressed by main neurons of the cerebellum, Purkinje cells (51). A model of congenital MCMV infection extrapolated significant data of MCMV involvement in neuronal impairment and altered development of mouse cerebellum (24, 52). As described by Koontz et al., MCMV infection in developing CNS demonstrates huge developmental abnormalities of the infected cerebellum in terms of delayed migration of postmitotic neurons from EGL into IGL, impaired morphology of Purkinje cells and decreased cerebellar area as compared to uninfected mice (24) (Figure 1B, C, D). Disruption of proliferation and differentiation of granule cells in the EGL of MCMV-infected newborn mice is manifested with decreased expression of TAG-1 (contactin-3) molecule and reduced expression of  $\alpha$  subunit of GABA A receptor (GABRA). Consequently, migration of granule cells is impaired and delayed lamination of cerebellar folia is observed (24). Purkinje cells present with impaired arborization of its dendrites and misslocalization outside of stratum gangliosum of developing cerebellar cortex. Studies on transgenic mice revealed active spreading of the Purkinje cell somas from a multilayered to a monolayer structure, and the orientation of dendrites towards the pial surface with the outgrowth of its dendritic branches is highly dependent on the function and maturation of granule cells during postnatal development of mouse cerebellum (53, 54). Observed impairment in differentiation of granule neurons in MCMV-infected newborn mice obviously influences the maturation of Purkinje cells as well. Infected newborn mice also exhibit a decreased expression of HOXA5, a CNS patterning transcription factor of the hindbrain region and decreased expression of TrkB, a high affinity receptor for BDNF (brain derived neurotrophic factor), a neurotrophin molecule that is involved in the promotion of neuronal survival, as well as the regulation of both pre-

and postnatal development of the cerebellum (24). These impairments result in a long term sequelae that are manifested as reduced performance on a balance beam behavioral assessment and profound sensorineural hearing loss in adult mice (unpublished data).

## IMMUNE RESPONSE TO CMV INFECTION OF THE CNS

Components of both cellular and humoral immune response are involved in the control of CMV infection. While CD8<sup>+</sup> lymphocytes T have the main role in the control of acute CMV infection, antibodies are essential for protection from reactivation and virus dissemination from latency (55, 56). CMV-specific antiviral response in the CNS was mainly studied on the model of MCMV in-



**Figure 2.** MCMV-induced encephalitis in newborn mouse brain. (A) MCMV-infected cells can be detected in brains of infected newborn mice. Immunohistochemical staining with MCMV IE1-specific MAbs reactive with MCMV IE1 protein is shown. Infection induces development of encephalitis characterized with (B) mononuclear cell infiltration (Cresyl violet staining). Immunophenotypic analysis of mononuclear cells infiltrations in infected mouse brain reveals that (C, D) CD4<sup>+</sup> T cells represent a minor immune population in MCMV-infected newborn brain, while (E, F) CD8<sup>+</sup> T cell predominate in infected CNS. Magnifications 20x (C, E) and 40x (A, B). Figure A presents the hippocampus of 9 day old infected newborn mice. Figures B – F presented brains from mice on post natal day 17. For details of experimental procedure see the reference (24, 58).

fection in newborn, or in adult immunodeficient mice and involves activation of both innate and adaptive immunity (24, 52, 57, 58). In brains of MCMV-infected newborn mice increased levels of: type I Interferons, number of proinflammatory chemokines (for example Interferon stimulated genes *Ifit1* and 3, *ISG12*, *ISG15*, members of *LY6* family *Ly6A/E* molecules), toll like receptors (especially *TLR-2*), *TNF- $\alpha$*  and *STAT-2* genes are observed; as well as increased expression *CXCL9* and *CXCL10* cytokines and *MHC class I* molecules (24). This is followed by the activation of both innate and adaptive cellular immune response comprised of NK cells, macrophages and lymphocytes T (24, 58, 59) (Figure 2).

Both astrocytes and microglia, the resident cells of the CNS, are activated following infection in the brain parenchyma. *In vitro* MCMV replication in astrocytes is inhibited by *TNF- $\alpha$* , *IL-1b* and *IFN- $\gamma$* , while microglia cells can support productive MCMV infection (60). Activated microglia and NK cells are detected in CMV-infected newborn brain (24, 58). Microglia cells function as an intrinsic immune system of the brain, and are considered to play a major role in traumatic lesions, neurodegenerative and infectious diseases in which survival of neurons is compromised (61). During MCMV infection of the CNS resident microglia cells secrete numbers of proinflammatory cytokines and chemokines in order to control the infection (62, 63). However there are evidence for the role of this immune response in the developmental impairment in infected CNS: *TNF- $\alpha$*  and *IL-1 $\beta$*  are shown to be implicated in neuronal damage (64, 65). Interestingly, *in vitro* infection of astrocyte cultures induced secretion of the most potent anti-inflammatory cytokine *TGF- $\beta$*  (66). NK cells are primary cells that control acute MCMV infection in adult mice (67), and although detectable in the CNS of MCMV-infected newborn mice, their role in the control of MCMV infection in the CNS is still insufficiently defined. *CD8<sup>+</sup>* T lymphocytes are major players that control MCMV infection in newborn brain (58). *CD8<sup>+</sup>* T cells present an effector phenotype by secreting *IFN- $\gamma$* , and infiltration of these cells into the CNS results in the termination of productive MCMV infection in brain parenchyma. While depletion of *CD8<sup>+</sup>* T lymphocytes in MCMV-infected pups is associated with 100% mortality, these cells show protective capacity onto virus replication even in adult immunocompromised mice acutely infected with MCMV (58). Conversely, in the brain of MCMV-infected adult, immunocompromised mice *CD4<sup>+</sup>* T lymphocytes were identified as key cells that reduce the amount CMV infection (57). Both *CD4<sup>+</sup>* and *CD8<sup>+</sup>* T cells isolated from infected brain express high levels of *PD-1* molecule, a member of *CD28* family of ligands that negatively regulates *CD8<sup>+</sup>* T cell function. Engagement of *PD-1* to its ligands, *PD-L1* and *PD-L2*, inhibits T cell proliferation and cytokine production and is shown to be upregulated on *CD8<sup>+</sup>* T cells during other chronic infections like LCMV or HIV (68, 69).

## TREATMENT AND PREVENTION OF CONGENITAL CMV INFECTION

Immunocompromised transplant recipients, HIV-infected patients and fetuses are at high risk of developing a serious and life-threatening CMV disease. Among these the incidence of severe manifestations of CMV infection are most prominent in infected infants. Children suffering acute HCMV infection in high percentage develop widespread encephalitis and cerebellar maldevelopment in terms of migration deficits of developing neurons and impairment in foliation of cerebellar cortex. Although preconceptual maternal immunity provides partial protection against CMV infection of the fetus (9, 70), high rate of congenital CMV infection is still a major public health problem. The highest rate of CMV transmission from mother to child is during primary maternal infection, although transplacental transmission of the virus can be seen in pregnant women with already established preconceptual immunity. This setting is observed in seropositive women infected with different CMV strains (4, 71). Data regarding the role of preconceptual immunity in the protection against congenital CMV infection and subsequent development of neurological sequelae are controversial. Some studies show that the presence of maternal antibodies is associated with decreased rate of congenital CMV infection and improved neurological outcome of infected infants (72, 73). Others studies show little, if any effect of maternal preconceptual immunity on protection against virus-induced neurologic damage in infected fetus following primary maternal infections, or the rate of neurological sequelae in congenitally infected infants (45, 74). Considering these data, treatment of congenital CMV infection is necessary. Currently, four drugs are licensed for use in treatment of systemic CMV infection, all belonging to inhibitors of viral DNA polymerase: ganciclovir, valganciclovir, foscarnet and cidofovir. From these, the first two are used in therapy of congenital CMV infection. Ganciclovir is the therapy of choice for severe CMV disease in immunocompromised adult patients but it is considered to be the best choice for treatment of severe CMV infection in newborns (75). Although data presenting efficacious postnatal therapy of congenital CMV infection with this drug regarding long term neurological development are faulty, recent study performed in Alabama, USA, showed that treatment of infected children with ganciclovir had a positive, protective impact on the cosequent development of hearing loss (76). Ganciclovir medication is mutagenic, teratogenic and carcinogenic, and there are still opposite standpoints of the benefit from this treatment. The pharmacokinetics of this drug and its success in the treatment of severe complications of CMV infection in immunocompromised adults makes it a novel candidate for use in the treatment of CMV infection in newborns (77).

Another approach of treatment in congenital HCMV infection is the passive immunization of pregnant women with antibodies specific for HCMV. Intravenous injection of HCMV hyperimmune globulin to pregnant women suffering acute HCMV infection showed the

protective effect on the rate of intrauterine transmission of HCMV from mother to the developing fetus (78). This protective effect of CMV immunoglobulins has still to be confirmed by additional clinical studies. In a murine model of perinatal MCMV infection we have shown that passive immunization of infected newborn mice with either immune serum or immunoglobulins specific for MCMV envelope glycoprotein gB reduces the rate of MCMV infection in developing brain and improves the neurological outcome in perinatally infected newborn mice (26). Virus replication in brains of newborn mice that received immune sera or antibodies specific for MCMV gB was decreased to non-detectable levels, which was accompanied with reduced amount of inflammation. Most importantly, parameters of postnatal cerebellum development were highly improved in these mice, as compared to controls (26).

Having in mind the high risk of infection in immunocompromised patients and infants, severity of sequelae following infection in these populations and the lack of efficacious and safe treatment, development of a CMV

vaccine has been declared as the highest priority in developed countries (79). So far, different immunization strategies have been used to develop an efficacious vaccine against CMV infection for use in these high-risk subjects. Development of a vaccine against congenital CMV infection has been hampered by the species specificity of the virus. This has precluded the evaluation of experimental vaccines against human CMV challenge in animal studies. Different CMV vaccines have been constructed: live attenuated vaccines, vectored vaccines using viral vectors, protein subunit vaccines; peptide vaccines and DNA vaccines, from which live attenuated vaccines and subunit vaccines have been tested in human trials (Table 1). First tested among live attenuated vaccines was AD169 strain of the HCMV which showed to induce virus-specific humoral response, but no clinical trials have been proceeded. Live attenuated Towne virus as a vaccine candidate, on the other hand, is extensively used in human clinical trials. Infection with this virus induces both CD8<sup>+</sup> T cell mediated immune response and generation of virus-neutralizing antibodies at levels comparable to those induced in natural HCMV infection (80,

TABLE 1

Designed CMV vaccines.

Vaccine	Trial results (references)	
Live attenuated vaccines	Towne vaccine	inefficient in prevention of CMV infection following transplantation or transplacental infection (83)
	Towne/Toledo vaccines	well tolerated, ongoing studies in seronegative subjects (100)
	AD169 vaccine	first tested, induces HCMV-specific antibody response (101)
Subunit vaccines «vectored» vaccines	gB/canarypox vectored vaccine (ALVAC)	characterized as suboptimal immunogenic (94, 95)
	gB/ie1/vaccinia Ankara vectored vaccine (MVA)	not tested in human trials, induces humoral immunity in a murine model (102)
	pp65/canarypox vectored vaccine	induces both humoral and cellular immune response (96)
	pp65/alphavirus vectored vaccine	not tested in human trials (99)
	gM/gN (gcII complex)	not tested in human trials (103)
	gH/gL/gO (gcIII complex)	not tested in human trials (103)
DNA vaccines	gB/pp65 bivalent DNA vaccine	ongoing studies in hematopoietic stem cell transplant patients (104)
	gB/pp65/ie1 trivalent DNA vaccine	As bivalent DNA vaccine, formulated using polomaxer adjuvant CRL1005 and benzalkonium chloride Induces T cell response against live attenuated CMV (Towne) (105–107)
Adjuvanted protein vaccines	gB/M59 adjuvant	ongoing studies in seronegative women, induces both humoral and cellular immune response, good safety profile (90, 91, 108)
Preclinical vaccine approaches		
Dense body vaccines	enveloped, replication-defective particles formed during CMV replication in cell cultures	induce both humoral and cellular immune response in murine models (109)
Nonstructural genes	DNA polymerase and helicase genes	tested in murine models elicits strong humoral and cellular immune response (110)
Bacterial artificial chromosomes	recombinant vaccines with specific genomic deletions or insertions which can improve the safety profile of a candidate vaccine or modify immune response	not tested in human trials, induces T cell response in guinea pig model (111)
»Prime boost« approach	priming with DNA vaccine is followed by boosting with formalin-inactivated viral particles	induces both humoral and CD8 <sup>+</sup> T cell immune response in animal models (103, 104)

81). Consequently, vaccination with this virus reduced the risk of CMV infection, as well as the incidence of severe CMV-induced disease in seronegative transplant recipients and increased the probability of a graft acceptance in transplant patients (82). Towne virus does not reactivate even in immunocompromised patients, does not shed and does not produce viremia. However, in early studies this vaccine did not prevent infection of mothers of children excreting CMV (83).

In protein subunit vaccines, CMV-main immunogenic proteins are incorporated into a viral vector in order to stimulate both humoral and cell-mediated immune responses (84). Numerous CMVs antigens (peptides) are characterized as capable to elicit strong specific T cell response (85, 86). One of the first attempts using peptides as vaccine candidates was an isolation of nine amino-acid T-cell epitope and spliced into a vaccinia virus vector conjugated with hepatitis B core protein (87). Vaccination with this virus elicited CD8<sup>+</sup> T lymphocyte antiviral response and protected against lethal disease in a murine model; however it failed to induce humoral immunity. Among protein subunit vaccines the best prospect for antiviral activity has the CMV immunodominant glycoprotein gB combined with adjuvant (MF59) or expressed in cell lines. CMV infection typically induces a serum antibody response to glycoprotein B (88) and crucial epitopes for virus neutralization are contained within conserved regions of this protein (89). Monoclonal antibodies specific for gB neutralize both wild type viral isolates and laboratory viral strains. In human trials, vaccine containing gB coupled with MF59 adjuvant induced high levels of antibody response, and interestingly this effect was more prominent in infants than in adults (90). Most importantly, recent data presented decreased incidence of maternal and congenital CMV infection in women receiving vaccine containing glycoprotein gB coupled with MF59 adjuvant (91).

Another CMV protein that belongs to viral tegument, pp65 has been used as a candidate for efficient CMV subunit vaccine based on the findings of its strongest induction of the CD8<sup>+</sup> T lymphocyte response (92, 93). Both gB and pp65 have been expressed in a recombinant canarypox ALVAC system (attenuated poxvirus which replicates productively in avian species but abortively in mammalian cells) and are shown to be immunogenic and well tolerated (94–96). ALVAC expressing gB has been tested in human trials and showed similar results in the induction of antibody and cell-mediated immune responses as compared to humans vaccinated with CMV gB/MF59 vaccine (95), while ALVAC expressing pp65 has showed to elicit CMV specific CD8<sup>+</sup> T cell response in CMV seronegative adults (96). Additional CMV vectored vaccines utilize modified vaccinia virus and Venezuelan equine encephalitis virus as vectors for either gB or pp65 CMV proteins. These vaccines are predominantly tested in animal models. In MCMV model vaccinia recombinant expressing gB, when administered as a vaccine, protected animals from lethal MCMV challenge (97). In guinea pig CMV (GPCMV) model gB vac-

cine partially protected pups from congenital GPCMV infection and in cases when the infection occurred, the viral DNA load was decreased in pups born from gB-vaccinated mothers, as compared to controls (98). In the same model, vaccination of dams with GP83 homolog of the HCMV pp65 phosphoprotein coupled with Venezuelan equine encephalitis virus followed by early trimester GPCMV challenge resulted in improved pregnancy outcome and reductions in maternal blood viral load (99). However, these vaccines have not yet been tested in human trials. Ongoing studies are performed using dense body vaccines, vaccines with nonstructural genes, bacterial artificial chromosomes and »prime boost« approach (Table 1). Until rationale design of such products and preclinical testing in animal models is accomplished, a strategy for development and testing that is focused on human studies seems inherently flawed. We would propose using preclinical studies in relevant animal models and a realistic goal – the generation of protective responses either by induction and/or transfer of immunity that can protect the CNS from damaging infection. This should be the primary goal for prevention of significant disease from congenital HCMV infection.

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## REFERENCES

1. PASS R F 2001 *Cytomegalovirus*, in Fields Virology, D.M. Knipe and P.M. Howley, Editors., Lippincott Williams and Wilkins, Philadelphia, p 2675–2706
2. ALFORD C A, BRITT W J 1990 *Cytomegalovirus*, in Virology, B.N. Fields and D.M. Knipe, Editors., Raven Press, New York, NY, p 1981–2010
3. NELSON C T, DEMMLER G J 1997 Cytomegalovirus infection in the pregnant mother, fetus, and newborn infant. *Clin Perinatol* 24(1): 151–60
4. BOPANA S B, RIVERA L B, FOWLER K B, MACH M, BRITT W J 2001 Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. *N Engl J Med* 344 (18): 1366–71
5. YINON Y, FARINE D, YUDIN M H, GAGNON R, HUDON L, BASSO M, BOS H, DELISLE M F, MENTICOGLU S, MUNDLE W, OUELLET A, PRESSEY T, ROGGENSACK A, BOUCHER M, CASTILLO E, GRUSLIN A, MONEY D M, MURPHY K, OGILVIE G, PAQUET C, VAN EYK N, VAN SCHALKWYK J 2010 Cytomegalovirus infection in pregnancy. *J Obstet Gynaecol Can* 32(4): 348–54
6. BALE J F, JR 1984 Human cytomegalovirus infection and disorders of the nervous system. *Arch Neurol* 41(3): 310–20
7. BARKOVICH A J, LINDAN C E 1994 Congenital cytomegalovirus infection of the brain: imaging analysis and embryologic considerations. *AJNR Am J Neuroradiol* 15(4): 703–15
8. STAGNO S, PASS R F, CLOUD G, BRITT W J, HENDERSON R E, WALTON P D, VEREN D A, PAGE F, ALFORD C A 1986 Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome. *JAMA* 256(14): 1904–8
9. FOWLER K B, STAGNO S, PASS R F, BRITT W J, BOLL T J, ALFORD C A 1992 The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. *N Engl J Med* 326(10): 663–7
10. STARATTON K, DURCH J, LAWRENCE R 2001 *Vaccines for the 21st century: a tool for decisionmaking*. Vol. 1. Nat Acad Press, Washington DC.



11. FRITSCHY J M, BRANDNER S, AGUZZI A, KOEDOOD M, LUSCHER B, MITCHELL P J 1996 Brain cell type specificity and gliosis-induced activation of the human cytomegalovirus immediate-early promoter in transgenic mice. *J Neurosci* 16(7): 2275–82
12. POWER C, POLAND S D, BLUME W T, GIRVIN J P, RICE G P 1990 Cytomegalovirus and Rasmussen's encephalitis. *Lancet* 336(8726): 1282–4
13. SALAZARA, PODZAMCZER D, RENE R, SANTIN M, PEREZ J L, FERRER I, FERNANDEZ-VILADRICH P, GUDIOL F 1995 Cytomegalovirus ventriculoencephalitis in AIDS patients. *Scand J Infect Dis* 27(2): 165–9
14. REUTER J D, GOMEZ D L, WILSON J H, VAN DEN POL A N 2004 Systemic immune deficiency necessary for cytomegalovirus invasion of the mature brain. *J Virol* 78(3): 1473–87
15. SAEDERUP N, LIN Y C, DAIRAGHI D J, SCHALL T J, MOCARSKI E S 1999 Cytomegalovirus-encoded beta chemokine promotes monocyte-associated viremia in the host. *Proc Natl Acad Sci U S A* 96(19): 10881–6
16. BENTZ G L, JARQUIN-PARDO M, CHAN G, SMITH M S, SINZGER C, YUROCHKO A D 2006 Human cytomegalovirus (HCMV) infection of endothelial cells promotes naive monocyte extravasation and transfer of productive virus to enhance hematogenous dissemination of HCMV. *J Virol* 80(23): 11539–55
17. FISH K N, SODERBERG-NAUCLER C, MILLS L K, STENGLIN S, NELSON J A 1998 Human cytomegalovirus persistently infects aortic endothelial cells. *J Virol* 72(7): 5661–8
18. ARGYRIS E G, ACHEAMPONG E, NUNNARI G, MUKHTAR M, WILLIAMS K J, POMERANTZ R J 2003 Human immunodeficiency virus type 1 enters primary human brain microvascular endothelial cells by a mechanism involving cell surface proteoglycans independent of lipid rafts. *J Virol* 77(22): 12140–51
19. COSBY S L, BRANKIN B 1995 Measles virus infection of cerebral endothelial cells and effect on their adhesive properties. *Vet Microbiol* 44(2–4): 135–9
20. LATHEY J L, WILEY C A, VERITY M A, NELSON J A 1990 Cultured human brain capillary endothelial cells are permissive for infection by human cytomegalovirus. *Virology* 176(1): 266–73
21. LUABEYA M K, DALLASTA L M, ACHIM C L, PAUZA C D, HAMILTON R L 2000 Blood-brain barrier disruption in simian immunodeficiency virus encephalitis. *Neuropathol Appl Neurobiol* 26(5): 454–62
22. DALLASTA L M, PISAROV L A, ESPLIN J E, WERLEY J V, MOSES A V, NELSON J A, ACHIM C L 1999 Blood-brain barrier tight junction disruption in human immunodeficiency virus-1 encephalitis. *Am J Pathol* 155(6): 1915–27
23. ANDRAS I E, PU H, DELI M A, NATH A, HENNIG B, TOBOREK M 2003 HIV-1 Tat protein alters tight junction protein expression and distribution in cultured brain endothelial cells. *J Neurosci Res* 74(2): 255–65
24. KOONTZ T, BRALIC M, TOMAC J, PERNJAK-PUGEL E, BANTUG G, JONJIC S, BRITT W J 2008 Altered development of the brain after focal herpesvirus infection of the central nervous system. *J Exp Med* 205(2): 423–35
25. VORBRODT A W, DOBROGOWSKA D H, KOZLOWSKI P, TARNAWSKI M, DUMAS R, RABE A 2001 Effect of a single embryonic exposure to alcohol on glucose transporter (GLUT-1) distribution in brain vessels of aged mouse. *J Neurocytol* 30(2): 167–74
26. CEKINOVIC D, GOLEMACE M, PUGEL E P, TOMAC J, CINCIN-SAIN L, SLAVULJICA I, BRADFORD R, MISCH S, WINKLER T H, MACH M, BRITT W J, JONJIC S 2008 Passive immunization reduces murine cytomegalovirus-induced brain pathology in newborn mice. *J Virol* 82(24): 12172–80
27. LUO M H, SCHWARTZ P H, FORTUNATO E A 2008 Neonatal neural progenitor cells and their neuronal and glial cell derivatives are fully permissive for human cytomegalovirus infection. *J Virol* 82(20): 9994–10007
28. KOSUGI I, SHINMURA Y, KAWASAKI H, ARAI Y, LI R Y, BABA S, TSUTSUI Y 2000 Cytomegalovirus infection of the central nervous system stem cells from mouse embryo: a model for developmental brain disorders induced by cytomegalovirus. *Lab Invest* 80(9): 1373–83
29. SHINMURA Y, AIBA-MASAGO S, KOSUGI I, LI R Y, BABA S, TSUTSUI Y 1997 Differential expression of the immediate-early and early antigens in neuronal and glial cells of developing mouse brains infected with murine cytomegalovirus. *Am J Pathol* 151(5): 1331–40
30. LOKENSGARD J R, CHEERAN M C, GEKKER G, HU S, CHAO C C, PETERSON P K 1999 Human cytomegalovirus replication and modulation of apoptosis in astrocytes. *J Hum Virol* 2(2): 91–101
31. POLAND S D, COSTELLO P, DEKABAN G A, RICE G P 1990 Cytomegalovirus in the brain: in vitro infection of human brain-derived cells. *J Infect Dis* 162(6): 1252–62
32. PULLIAM L 1991 Cytomegalovirus preferentially infects a monocyte derived macrophage/microglial cell in human brain cultures: neuropathology differs between strains. *J Neuropathol Exp Neurol* 50(4): 432–40
33. BECROFT D M O 1981 *Prenatal cytomegalovirus infection: epidemiology, pathology and pathogenesis*, in Perspective in pediatric pathology, In: R. H.S. and B. J. (eds). Masson Press, New York, p 203–241
34. PERLMAN J M, ARGYLE C 1992 Lethal cytomegalovirus infection in preterm infants: clinical, radiological, and neuropathological findings. *Ann Neurol* 31(1): 64–8
35. MARQUES DIAS M J, HARMANT-VAN RIJCKEVORSEL G, LANDRIEU P, LYON G 1984 Prenatal cytomegalovirus disease and cerebral microgyria: evidence for perfusion failure, not disturbance of histogenesis, as the major cause of fetal cytomegalovirus encephalopathy. *Neuropediatrics* 15(1): 18–24
36. DE VRIES L S, GUNARDI H, BARTH P G, BOK L A, VERBOON-MACIOLEK M A, GROENENDAAL F 2004 The spectrum of cranial ultrasound and magnetic resonance imaging abnormalities in congenital cytomegalovirus infection. *Neuropediatrics* 35(3): 113–9
37. STEINLIN M I, NADAL D, EICH G F, MARTIN E, BOLTSCHAUER E J 1996 Late intrauterine Cytomegalovirus infection: clinical and neuroimaging findings. *Pediatr Neurol* 15(3): 249–53
38. SUGITA K, ANDO M, MAKINO M, TAKANASHI J, FUJIMOTO N, NIIMI H 1991 Magnetic resonance imaging of the brain in congenital rubella virus and cytomegalovirus infections. *Neuroradiology* 33(3): 239–42
39. BOESCH C, ISSAKAINEN J, KEWITZ G, KIKINIS R, MARTIN E, BOLTSCHAUER E 1989 Magnetic resonance imaging of the brain in congenital cytomegalovirus infection. *Pediatr Radiol* 19(2): 91–3
40. TRINCADO D E, RAWLINSON W D 2001 Congenital and perinatal infections with cytomegalovirus. *J Paediatr Child Health* 37(2): 187–92
41. VAN DER KNAAP M S, VERMEULEN G, BARKHOF F, HART A A, LOEBER J G, WEEL J F 2004 Pattern of white matter abnormalities at MR imaging: use of polymerase chain reaction testing of Guthrie cards to link pattern with congenital cytomegalovirus infection. *Radiology* 230(2): 529–36
42. DAHLE A J, FOWLER K B, WRIGHT J D, BOPANA S B, BRITT W J, PASS R F 2000 Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus. *J Am Acad Audiol* 11(5): 283–90
43. HICKS T, FOWLER K, RICHARDSON M, DAHLE A, ADAMS L, PASS R 1993 Congenital cytomegalovirus infection and neonatal auditory screening. *J Pediatr* 123(5): 779–82
44. FOWLER K B, PASS R F 2006 Risk factors for congenital cytomegalovirus infection in the offspring of young women: exposure to young children and recent onset of sexual activity. *Pediatrics* 118(2): e286–92
45. ROSS S A, FOWLER K B, ASHRITH G, STAGNO S, BRITT W J, PASS R F, BOPANA S B 2006 Hearing loss in children with congenital cytomegalovirus infection born to mothers with preexisting immunity. *J Pediatr* 148(3): 332–6
46. VAN DEN POL A N, MOCARSKI E, SAEDERUP N, VIEIRA J, MEIER T J 1999 Cytomegalovirus cell tropism, replication, and gene transfer in brain. *J Neurosci* 19(24): 10948–65
47. CHAUDHURI S, LOWEN B, CHAN G, DAVEY A, RIDDELL M, GUILBERT L J 2009 Human cytomegalovirus interacts with toll-like receptor 2 and CD14 on syncytiotrophoblasts to stimulate expression of TNF $\alpha$  mRNA and apoptosis. *Placenta* 30(11): 994–1001
48. ADLER S P, NIGRO G, PEREIRA L 2007 Recent advances in the prevention and treatment of congenital cytomegalovirus infections. *Semin Perinatol* 31(1): 10–8
49. RAWLINSON W D, FARRELL H E, BARRELL B G 1996 Analysis of the complete DNA sequence of murine cytomegalovirus. *J Virol* 70(12): 8833–49

50. KRMPOTIC A, BUBIC I, POLIC B, LUCIN P, JONJIC S 2003 Pathogenesis of murine cytomegalovirus infection. *Microbes Infect* 5(13): 1263–77
51. BEHESTI H, MARINO S 2009 Cerebellar granule cells: insights into proliferation, differentiation, and role in medulloblastoma pathogenesis. *Int J Biochem Cell Biol* 41(3): 435–45
52. TSUTSUI Y, KOSUGI I, KAWASAKI H 2005 Neuropathogenesis in cytomegalovirus infection: indication of the mechanisms using mouse models. *Rev Med Virol* 15(5): 327–45
53. RAKIC P, SIDMAN R L 1973 Organization of cerebellar cortex secondary to deficit of granule cells in weaver mutant mice. *J Comp Neurol* 152(2): 133–61
54. ROSS M E, FLETCHER C, MASON C A, HATTEN M E, HEINTZ N 1990 Meander tail reveals a discrete developmental unit in the mouse cerebellum. *Proc Natl Acad Sci U S A* 87(11): 4189–92
55. JONJIC S, PAVIC I, POLIC B, CRNKOVIC I, LUCIN P, KOSZINOWSKI U H 1994 Antibodies are not essential for the resolution of primary cytomegalovirus infection but limit dissemination of recurrent virus. *J Exp Med* 179(5): 1713–7
56. POLIC B, HENGEL H, KRMPOTIC A, TRGOVICICH J, PAVIC I, LUCCARONIN P, JONJIC S, KOSZINOWSKI U H 1998 Hierarchical and redundant lymphocyte subset control precludes cytomegalovirus replication during latent infection. *J Exp Med* 188(6): 1047–54
57. REUTER J D 2005 Cytomegalovirus induces T-cell independent apoptosis in brain during immunodeficiency. *J Clin Virol* 32(3): 218–23
58. BANTUG G R, CEKINOVIC D, BRADFORD R, KOONTZ T, JONJIC S, BRITT W J 2008 CD8+ T lymphocytes control murine cytomegalovirus replication in the central nervous system of newborn animals. *J Immunol* 181(3): 2111–23
59. CHEERAN M C, GEKKER G, HU S, MIN X, COX D, LOKENSGARD J R 2004 Intracerebral infection with murine cytomegalovirus induces CXCL10 and is restricted by adoptive transfer of splenocytes. *J Neurovirol* 10(3): 152–62
60. CHEERAN M C, HU S, GEKKER G, LOKENSGARD J R 2000 Decreased cytomegalovirus expression following proinflammatory cytokine treatment of primary human astrocytes. *J Immunol* 164(2): 926–33
61. MINAGARA, SHAPSHAK P, FUJIMURA R, OWNBY R, HEYES M, EISDORFER C 2002 The role of macrophage/microglia and astrocytes in the pathogenesis of three neurologic disorders: HIV-associated dementia, Alzheimer disease, and multiple sclerosis. *J Neurol Sci* 202(1–2): 13–23
62. CHEERAN M C, HU S, SHENG W S, PETERSON P K, LOKENSGARD J R 2003 CXCL10 production from cytomegalovirus-stimulated microglia is regulated by both human and viral interleukin-10. *J Virol* 77(8): 4502–15
63. LOKENSGARD J R, HU S, SHENG W, VANOIJEN M, COX D, CHEERAN M C, PETERSON P K 2001 Robust expression of TNF-alpha, IL-1beta, RANTES, and IP-10 by human microglial cells during nonproductive infection with herpes simplex virus. *J Neurovirol* 7(3): 208–19
64. STANLEY L C, MRAK R E, WOODY R C, PERROT L J, ZHANG S, MARSHAK D R, NELSON S J, GRIFFIN W S 1994 Glial cytokines as neuropathogenic factors in HIV infection: pathogenic similarities to Alzheimer's disease. *J Neuropathol Exp Neurol* 53(3): 231–8
65. GRIFFIN D E 1997 Cytokines in the brain during viral infection: clues to HIV-associated dementia. *J Clin Invest* 100(12): 2948–51
66. KOSSMANN T, MORGANTI-KOSSMANN M C, ORENSTEIN J M, BRITT W J, WAHL S M, SMITH P D 2003 Cytomegalovirus production by infected astrocytes correlates with transforming growth factor-beta release. *J Infect Dis* 187(4): 534–41
67. JONJIC S, BUBIC I, KRMPOTIC A 2006 *Innate Immunity to Cytomegaloviruses*, in *Cytomegaloviruses: Molecular Biology and Immunology*, In: Reddehase M J (ed). Caister Academic Press, p 285–319
68. BLATTMAN J N, WHERRY E J, HA S J, VAN DER MOST R G, AHMED R 2009 Impact of epitope escape on PD-1 expression and CD8 T-cell exhaustion during chronic infection. *J Virol* 83(9): 4386–94
69. VELU V, KANNANGANAT S, IBEGBU C, CHENNAREDDI L, VILLINGER F, FREEMAN G J, AHMED R, AMARA R R 2007 Elevated expression levels of inhibitory receptor programmed death 1 on simian immunodeficiency virus-specific CD8 T cells during chronic infection but not after vaccination. *J Virol* 81(11): 5819–28
70. ADLER S P, STARR S E, PLOTKIN S A, HEMPFLING S H, BUIS J, MANNING M L, BEST A M 1995 Immunity induced by primary human cytomegalovirus infection protects against secondary infection among women of childbearing age. *J Infect Dis* 171(1): 26–32
71. BOPPANA S B, FOWLER K B, BRITT W J, STAGNO S, PASS R F 1999 Symptomatic congenital cytomegalovirus infection in infants born to mothers with preexisting immunity to cytomegalovirus. *Pediatrics* 104(1 Pt 1): 55–60
72. FOWLER K B, STAGNO S, PASS R F 2003 Maternal immunity and prevention of congenital cytomegalovirus infection. *Jama* 289(8): 1008–11
73. EGGERS M, RADSAK K, ENDERS G, RESCHKE M 2001 Use of recombinant glycoprotein antigens gB and gH for diagnosis of primary human cytomegalovirus infection during pregnancy. *J Med Virol* 63(2): 135–42
74. BOPPANA S B, POLIS M A, KRAMER A A, BRITT W J, KOENIG S 1995 Virus-specific antibody responses to human cytomegalovirus (HCMV) in human immunodeficiency virus type 1-infected persons with HCMV retinitis. *J Infect Dis* 171(1): 182–5
75. DEVRIES J 2007 The ABCs of CMV. *Adv Neonatal Care* 7(5): 248–55; quiz 256–7
76. KIMBERLIN D W, LIN C Y, SANCHEZ P J, DEMMLER G J, DANKNER W, SHELTON M, JACOBS R F, VAUDRY W, PASS R F, KIELL J M, SOONG S J, WHITLEY R J 2003 Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: a randomized, controlled trial. *J Pediatr* 143(1): 16–25
77. KIMBERLIN D W 2002 Antiviral therapy for cytomegalovirus infections in pediatric patients. *Semin Pediatr Infect Dis* 13(1): 22–30
78. NIGRO G, ADLER S P, LA TORRE R, BEST A M 2005 Passive immunization during pregnancy for congenital cytomegalovirus infection. *N Engl J Med*. 353(13): 1350–62
79. STRATTON K R, DURCH J S, LAWRENCE R S 2001 *Vaccines for the 21st Century: A Tool for Decision Making*. National Academy Press, Washington DC.
80. ADLER S P, HEMPFLING S H, STARR S E, PLOTKIN S A, RIDDELL S 1998 Safety and immunogenicity of the Towne strain cytomegalovirus vaccine. *Pediatr Infect Dis J* 17(3): 200–6
81. JACOBSON M A, SINCLAIR E, BREDT B, AGRILLO L, BLACK D, EPLING C L, CARVIDI A, HO T, BAINS R, ADLER S P 2006 Antigen-specific T cell responses induced by Towne cytomegalovirus (CMV) vaccine in CMV-seronegative vaccine recipients. *J Clin Virol* 35(3): 332–7
82. STARR S E 1992 Cytomegalovirus vaccines: current status. *Infect Agents Dis* 1(3): 146–8
83. ADLER S P, FINNEY J W, MANGANELLO A M, BEST A M 1996 Prevention of child-to-mother transmission of cytomegalovirus by changing behaviors: a randomized controlled trial. *Pediatr Infect Dis J* 15(3): 240–6
84. DIAMOND D J, YORK J, SUN J Y, WRIGHT C L, FORMAN S J 1997 Development of a candidate HLA A\*0201 restricted peptide-based vaccine against human cytomegalovirus infection. *Blood* 90(5): 1751–67
85. MORELLO C S, CRANMER L D, SPECTOR D H 2000 Suppression of murine cytomegalovirus (MCMV) replication with a DNA vaccine encoding MCMV M84 (a homolog of human cytomegalovirus pp65). *J Virol* 74(8): 3696–708
86. MUNKS M W, GOLD M C, ZAJAC A L, DOOM C M, MORELLO C S, SPECTOR D H, HILL A B 2006 Genome-wide analysis reveals a highly diverse CD8 T cell response to murine cytomegalovirus. *J Immunol* 176(6): 3760–6
87. DEL VAL M, SCHLICHT H J, VOLKMER H, MESSERLE M, REDDEHASE M J, KOSZINOWSKI U H 1991 Protection against lethal cytomegalovirus infection by a recombinant vaccine containing a single nonameric T-cell epitope. *J Virol* 65(7): 3641–6
88. MARSHALL G S, RABALAIS G P, STOUT G G, WALDEYER S L 1992 Antibodies to recombinant-derived glycoprotein B after natural human cytomegalovirus infection correlate with neutralizing activity. *J Infect Dis*. 165(2): 381–4
89. MACH M 2006 *Antibody-mediated Neutralization of Infectivity*, in *Cytomegaloviruses: Molecular Biology and Immunology*, In: Reddehase M (ed) Caister Academic Press, p 265–283

90. MITCHELL D K, HOLMES S J, BURKE R L, DULIEGE A M, ADLER S P 2002 Immunogenicity of a recombinant human cytomegalovirus gB vaccine in seronegative toddlers. *Pediatr Infect Dis J* 21(2): 133–8
91. PASS R F, ZHANG C, EVANS A, SIMPSON T, ANDREWS W, HUANG M L, COREY L, HILL J, DAVIS E, FLANIGAN C, CLOUD G 2009 Vaccine prevention of maternal cytomegalovirus infection. *N Engl J Med* 360(12): 1191–9
92. MCLAUGHLIN-TAYLOR E, PANDE H, FORMAN S J, TANAMACHI B, LI C R, ZAIA J A, GREENBERG P D, RIDDELL S R 1994 Identification of the major late human cytomegalovirus matrix protein pp65 as a target antigen for CD8+ virus-specific cytotoxic T lymphocytes. *J Med Virol* 43(1): 103–10
93. WILLS M R, OKECHA G, WEEKES M P, GANDHI M K, SISSONS P J, CARMICHAEL A J 2002 Identification of naive or antigen-experienced human CD8(+) T cells by expression of costimulation and chemokine receptors: analysis of the human cytomegalovirus-specific CD8(+) T cell response. *J Immunol*. 168(11): 5455–64
94. ADLER S P, PLOTKIN S A, GONCZOL E, CADOZ M, MERIC C, WANG J B, DELLAMONICA P, BEST A M, ZAHRADNIK J, PINCUS S, BERENCSI K, COX W I, GYULAI Z 1999 A canarypox vector expressing cytomegalovirus (CMV) glycoprotein B primes for antibody responses to a live attenuated CMV vaccine (Towne). *J Infect Dis*. 180(3): 843–6
95. BERNSTEIN D I, SCHLEISS M R, BERENCSI K, GONCZOL E, DICKEY M, KHOURY P, CADOZ M, MERIC C, ZAHRADNIK J, DULIEGE A M, PLOTKIN S 2002 Effect of previous or simultaneous immunization with canarypox expressing cytomegalovirus (CMV) glycoprotein B (gB) on response to subunit gB vaccine plus MF59 in healthy CMV-seronegative adults. *J Infect Dis* 185(5): 686–90
96. BERENCSI K, GYULAI Z, GONCZOL E, PINCUS S, COX W I, MICHELSON S, KARI L, MERIC C, CADOZ M, ZAHRADNIK J, STARR S, PLOTKIN S 2001 A canarypox vector-expressing cytomegalovirus (CMV) phosphoprotein 65 induces long-lasting cytotoxic T cell responses in human CMV-seronegative subjects. *J Infect Dis* 183(8): 1171–9
97. RAPP M 1993 *in* Multidisciplinary approach to understanding cytomegalovirus disease, S. Michelson and S.A. Plotkin, Editors., Elsevier Science Publishers BV: Amsterdam
98. SCHLEISS M R, BOURNE N, BERNSTEIN D I 2003 Preconception vaccination with a glycoprotein B (gB) DNA vaccine protects against cytomegalovirus (CMV) transmission in the guinea pig model of congenital CMV infection. *J Infect Dis* 188(12): 1868–74
99. SCHLEISS M R, LACAYO J C, BELKAID Y, MCGREGOR A, STROUP G, RAYNER J, ALTERSON K, CHULAY J D, SMITH J F 2007 Preconceptual administration of an alphavirus replicon UL83 (pp65 homolog) vaccine induces humoral and cellular immunity and improves pregnancy outcome in the guinea pig model of congenital cytomegalovirus infection. *J Infect Dis* 195(6): 789–98
100. HEINEMAN T C, SCHLEISS M, BERNSTEIN D I, SPAETE R R, YAN L, DUKE G, PRICHARD M, WANG Z, YAN Q, SHARP M A, KLEIN N, ARVIN A M, KEMBLE G 2006 A phase 1 study of 4 live, recombinant human cytomegalovirus Towne/Toledo chimeric vaccines. *J Infect Dis* 193(10): 1350–60
101. NEFF B J, WEIBEL R E, BUYNACK E B, MCLEAN A A, HILLEMANN M R 1979 Clinical and laboratory studies of live cytomegalovirus vaccine Ad-169. *Proc Soc Exp Biol Med* 160(1): 32–7
102. WANG Z, LA ROSA C, MAAS R, LY H, BREWER J, MEKHOUBAD S, DAFTARIAN P, LONGMATE J, BRITT W J, DIAMOND D J 2004 Recombinant modified vaccinia virus Ankara expressing a soluble form of glycoprotein B causes durable immunity and neutralizing antibodies against multiple strains of human cytomegalovirus. *J Virol*. 78(8): 3965–76
103. CHEERAN M C, LOKENSGARD J R, SCHLEISS M R 2009 Neuropathogenesis of congenital cytomegalovirus infection: disease mechanisms and prospects for intervention. *Clin Microbiol Rev* 22(1): 99–126, Table of Contents
104. SCHLEISS M R 2009 Cytomegalovirus vaccines: at last, a major step forward. *Herpes* 15(3): 44–5
105. VILALTA A, MAHAJAN R K, HARTIKKA J, RUSALOV D, MARTIN T, BOZOUKOVA V, LEAMY V, HALL K, LALOR P, ROLLAND A, KASLOW D C 2005 I. Poloxamer-formulated plasmid DNA-based human cytomegalovirus vaccine: evaluation of plasmid DNA biodistribution/persistence and integration. *Hum Gene Ther* 16(10): 1143–50
106. WLOCH M K, SMITH L R, BOUTSABOULOY S, REYES L, HAN C, KEHLER J, SMITH H D, SELK L, NAKAMURA R, BROWN J M, MARBURY T, WALD A, ROLLAND A, KASLOW D, EVANS T, BOECKH M 2008 Safety and immunogenicity of a bivalent cytomegalovirus DNA vaccine in healthy adult subjects. *J Infect Dis* 197(12): 1634–42
107. JACOBSON M A, ADLER S P, SINCLAIR E, BLACK D, SMITH A, CHU A, MOSS R B, WLOCH M K 2009 A CMV DNA vaccine primes for memory immune responses to live-attenuated CMV (Towne strain). *Vaccine* 27(10): 1540–8
108. ZHANG C, PASS R F 2004 Detection of cytomegalovirus infection during clinical trials of glycoprotein B vaccine. *Vaccine* 23(4): 507–10
109. PEPPERL-KLINDWORTH S, FRANKENBERG N, PLACHTER B 2002 Development of novel vaccine strategies against human cytomegalovirus infection based on subviral particles. *J Clin Virol* 25 (Suppl 2): S75–85
110. MORELLO C S, KELLEY L A, MUNKS M W, HILL A B, SPECTOR D H 2007 DNA immunization using highly conserved murine cytomegalovirus genes encoding homologs of human cytomegalovirus UL54 (DNA polymerase) and UL105 (helicase) elicits strong CD8 T-cell responses and is protective against systemic challenge. *J Virol* 81(14): 7766–75
111. SCHLEISS M R 2008 Comparison of vaccine strategies against congenital CMV infection in the guinea pig model. *J Clin Virol* 41(3): 224–30