

Treatment of congenital cytomegalovirus infection: implications for future therapeutic strategies

Lauren Nassetta¹, David Kimberlin¹ and Richard Whitley^{1,2*}

¹Department of Pediatrics, The University of Alabama at Birmingham, Birmingham, AL, USA; ²Departments of Medicine, Microbiology and Neurosurgery, The University of Alabama at Birmingham, Birmingham, AL, USA

Cytomegalovirus (CMV) infection is the most common cause of congenital infection in the developed world, occurring in ~1% of all liveborns. Symptomatic disease occurs in 10% of all congenitally infected infants, resulting in a spectrum of clinical manifestations that include microcephaly, chorioretinitis, hepatosplenomegaly and sensorineural hearing loss, among others. Even those children who are asymptomatic at birth have a risk of hearing loss, with ~8% experiencing this sequela. Overall, congenital CMV infection accounts for one-third of all cases of sensorineural hearing loss. The economic burden of disease exceeds \$2 billion annually in the USA. Therefore, this infection has been the target for antiviral therapy. Studies performed by the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (CASG) have evaluated ganciclovir for the treatment of symptomatic congenital CMV infection with central nervous system involvement. In a randomized, controlled clinical trial of ganciclovir treatment (6 mg/kg iv every 12 h for 6 weeks) brainstem-evoked responses were utilized as the primary endpoint and demonstrated stabilization of hearing both at 6 months and >1 year. Treatment was associated with neutropenia in over 60% of treated patients. Since ganciclovir must be given intravenously, studies with its prodrug, valganciclovir, have been performed to assess pharmacokinetics and pharmacodynamics. Currently, a clinical trial of 6 weeks versus 6 months of valganciclovir is being performed by the CASG. Notably, only intravenous ganciclovir and orally administered valganciclovir have been used to treat congenital CMV infection. Hopefully, other drugs such as maribavir will be available for evaluation in this population.

Keywords: congenital infection, therapy, CMV

Background

Cytomegalovirus (CMV) is the most common congenital infection of the developed world, affecting ~1% of all infants born in the USA and slightly less of all infants born worldwide. It is the leading viral cause of mental retardation and the most frequent non-hereditary cause of sensorineural hearing loss worldwide. CMV is the largest and most complex member of the herpesvirus family that infects humans,¹ named for its cytopathic effect of producing enlarged cells with intranuclear and cytoplasmic inclusions, which often give the cells their classic 'owl's eye' appearance. While these cytomegalic cells were first discovered in the kidneys² of stillborn infants and, subsequently, in parotid glands,³ they can be found in virtually every cell type of humans.^{4–9} *In vitro*, CMV most readily infects human fibroblasts, with less affinity for other cell types. In contrast, human infection results in viral replication in a wide variety of cells.¹⁰

Epidemiology

Human CMV is highly species-specific, with humans being the only host. Furthermore, CMV has been found in every human population tested.^{11–13} The prevalence of infection is greater in developing countries and among lower socioeconomic groups of developed countries.¹⁴ Overall, the seroprevalence of infection varies between 65% and 90% among middle age adults in the USA, where primary CMV infection during pregnancy occurs in 2% of women of childbearing age who are in middle-to-higher socioeconomic groups and in 6% of women who are of lower socioeconomic background.¹⁵

Crowded living conditions, poor sanitation, sexual practices and increased exposure to infants and children all contribute to increasing rates of infection and a higher seroprevalence. Virus can be isolated from urine, saliva, cervical and vaginal secretions, semen, breast milk, tears, blood products and transplanted organs.^{16–20} Thus, close contact,

*Corresponding author. E-mail: Rwhitley@peds.uab.edu

allowing for direct or indirect person-to-person contact, spreads the infection.

Congenital infection

Newborn infection occurs as the consequence of one of three routes of transmission: (i) intrauterine; (ii) intrapartum; and (iii) post-natal (breast milk acquisition). Intrauterine infection is usually the result of a susceptible woman acquiring infection from a child in the family or day care environment early during her gestation.²¹ Infection of women both immediately prior to and during pregnancy produces a risk of congenital CMV infection.^{22,23} *In utero* transmission occurs secondary to primary maternal infection, as is the case with toxoplasmosis and rubella, and also in recurrent infections, including reinfection with a different strain of the virus²⁴ or reactivation of the latent virus.²⁵ Testing women for CMV during pregnancy is not helpful because a large percentage of women shed the virus during pregnancy and yet have infants who do not develop congenital infection.

Infants and children are important sources for the spread of CMV. Indeed, most CMV infection during pregnancy derives from maternal exposure to children and infants who are infected with the virus. Multiple studies in Sweden and the USA have shown that the rate of CMV infection is much higher in children who attend day care than those who do not.^{26–31} Many initially seronegative children become infected with CMV from their day care peers. CMV infection, then, is transmitted horizontally from child to child, most likely through the spread of saliva on hands and toys.^{32,33} These children then excrete large amounts of CMV for extended periods of time, exposing parents and other caregivers who may become pregnant.

The amount of maternal shedding of virus directly correlates with the risk of perinatal infection. Infected breast milk and exposure to CMV in the genital tract lead to high rates of peripartum and post-natal CMV transmission.¹⁹ Infants who breast feed from CMV-seropositive women have an estimated rate of infection between 39% and 59%.^{16,34} The risk is greater when the maternal viral load is higher than 7×10^3 genome equivalents/mL. Excretion of the virus in breast milk is greatest between 2 weeks and 2 months post-partum. Infected infants usually begin to excrete CMV between 3 weeks and 3 months after birth. Many of these infants excrete CMV chronically (for years), providing an opportunity to infect caretakers or others in contact with these children.

Though most infants contract CMV congenitally or perinatally, nosocomial infection is also an important source of CMV infection for infants. CMV infection should be considered as a risk after blood transfusion and organ transplantation.^{35–40} CMV can cause life-threatening infections in premature neonates and in haematopoietic stem cell transplant recipients.^{41–43} CMV in transfused blood can also cause serious illness in infants who are born to seronegative mothers and, therefore, do not have the protective antibodies directed against the virus.

Clinical presentation

Only 10% of all infants born in the USA with congenital CMV infection have symptomatic disease at birth.¹ Thus, ~90% of infected children have no evidence of clinical disease. While

these children generally have a better prognosis than the symptomatic children, they are at risk for hearing loss; thus, the impact of infection on their health and development is not insignificant.

Hearing loss is the most significant developmental abnormality in children with asymptomatic infection. One study found hearing loss in 7.2% of patients with asymptomatic infection.⁴⁴ In 50% of these children, the hearing loss was bilateral, and in 50%, it was progressive. The median age at first progression of hearing loss was 18 months. Eighteen percent of children had delayed onset of sensorineural hearing loss, with the median age of detection at 27 months.

Cumulative data suggest that CMV infection causes at least one-third of sensorineural hearing loss in young children.^{45–47} Thus, universal neonatal screening for hearing loss will miss the significant proportion of CMV-associated hearing loss that develops over time and, as such, newborn hearing screening cannot completely detect all sensorineural hearing loss in children.

In contrast to asymptotically infected babies, CMV-infected neonates who are born with signs of infection ('symptomatic congenital CMV disease') often have dramatic presentations. Babies with symptomatic congenital CMV disease can have sensorineural hearing loss, microcephaly, motor defects, mental retardation, chorioretinitis and dental defects. The signs and symptoms of congenital CMV infection and their frequency have been recently reviewed.⁶⁷

Roughly, half of the infants with symptoms of infection at birth have generalized CMV that involves many organ systems.^{48,49} The most strikingly affected are the central nervous system (CNS) and the reticuloendothelial system. Patients with generalized congenital CMV infection most commonly present with hepatomegaly, splenomegaly, microcephaly, jaundice and petechiae.⁴⁹ Thirty percent of patients with severe disease die of multiorgan dysfunction.⁵⁰

Hepatomegaly and splenomegaly are the most common findings on physical examination in neonates with symptomatic congenital CMV.⁵¹ Splenomegaly may be the only sign of infection, but is common to all congenital infections.^{48,51} Hepatomegaly may be striking at birth, but is also relatively non-specific and usually resolves after ~1 year of age. Cutaneous manifestations of congenital CMV infection include jaundice and a generalized petechial rash. Jaundice from CMV can sometimes be distinguished from physiological jaundice because it can begin on the first day of life and usually lasts longer than physiological jaundice.^{48,51} Fortunately, about half of the total bilirubin is the direct bilirubin component so, while total levels may be high, it is unusual for the indirect component to be high enough for exchange transfusion.

The generalized petechial rash of CMV is caused by thrombocytopenia.^{51,52} Platelet counts vary widely but usually range from 20000 to 60000 platelets/mm³, though even patients with normal platelet counts can have petechiae. The petechial rash develops within a few hours of birth and persists for 48 h to a few weeks after birth. The rash is also caused in part by the prolongation of normal fetal extramedullary haematopoiesis.

Microcephaly affected about one-half of all surviving patients with congenital CMV in one study, as defined as head circumference less than the 5th percentile for age or gestational age.⁴⁸ Microcephaly has been found to be the most specific predictor of mental retardation. Mental retardation can also be predicted by the presence of intracranial calcifications, which predict at least moderate and probably severe mental retardation.

Review

Congenital CMV infection also involves the eye. Chorioretinitis, strabismus and optic atrophy are the most common abnormalities.^{48,51,53} About 14% of patients with symptomatic congenital CMV have some degree of chorioretinitis.^{48,53} The central retinal lesions of CMV cannot be distinguished clinically from those of toxoplasmosis.^{53,54} Eye disease can often appear as strabismus prompting closer examination of the eye. Unlike congenital toxoplasma infection, however, the retinitis caused by CMV does not progress.⁵³

Very few children with symptomatic congenital CMV survive with normal intellect and hearing. One or more handicaps occur in almost 90% of the patients who survive with symptomatic congenital CMV infection.⁴⁸ Seventy percent of children with symptomatic infection have psychomotor retardation, usually accompanied by neurological complications and microcephaly. Hearing loss occurs in 50% of patients, with bilateral hearing loss in 67% and progressive hearing loss in 54%. Low IQ is associated with microcephaly at birth, development of neurological problems within the first year of life, ocular lesions and microcephaly that becomes apparent after birth.¹

Abnormal computed tomography (CT) scan findings within the first month of life seem to be the best predictor of adverse neurodevelopmental outcomes.⁵⁵ CT scan findings are abnormal in 70% of symptomatic children, with the most common abnormality being intracerebral calcifications. One study interpreted CT scan data from 56 children with symptomatic CMV infection and reported that only 29% of children with a normal study developed at least one neurological sequela. In contrast, almost 90% of children with abnormal studies had at least one neurological abnormality. Fifty-nine percent of children with abnormal studies had an IQ of <70, when compared with only one child with a normal CT scan.

CMV infection causes distinct CNS disease when it is acquired congenitally.¹ CNS disease is often an on-going process, causing progressive changes for years after birth.^{44,48,54,56,57} Infection can cause structural changes within the CNS such as periventricular calcifications, ventriculomegaly and loss of grey–white matter differentiation.^{55,58,59} There is often loss of normal brain architecture with loss of normal radial neuronal migration.⁶⁰ Cerebrospinal fluid findings in infected infants generally reveal increased protein and white cells. Autopsy results reveal inflammatory infiltrates within the brain parenchyma.⁵⁹ These changes vary widely with age of gestation at time of infection or reactivation of the virus. They also vary greatly in the degree of disability they cause the patients.

Therapeutics and clinical trials

Currently, there are four licensed drugs for the systemic treatment of CMV infection: ganciclovir, valganciclovir (oral prodrug of ganciclovir), cidofovir and foscarnet. In addition, fomivirsen is licensed for intravitreal administration to treat CMV retinitis in patients with AIDS. Ganciclovir is phosphorylated by UL97, a kinase unique to CMV replication. Ganciclovir and valganciclovir are the only two medications that have been employed in the treatment of congenital CMV infection to date, and are the focus of our review.

The National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (CASG) conducted a pharmacokinetic–pharmacodynamic study that established the safe

dose of intravenous ganciclovir to be administered to infected infants.⁶¹ This was followed by a Phase III randomized controlled study to determine the effects of ganciclovir therapy on hearing in the treatment of symptomatic congenital CMV disease involving the CNS.⁶²

In this Phase III study, 100 neonates, all <1 month of age, with symptomatic congenital CMV involving the CNS, as defined by microcephaly, intracranial calcifications, abnormal cerebrospinal fluid for age, chorioretinitis and/or hearing deficits with confirmed isolation of CMV from a urine specimen, were enrolled after parental consent. Infected newborns were randomized to receive either ganciclovir or no therapy. The patients in the ganciclovir treatment arm received 6 mg/kg per dose intravenously every 12 h for 6 weeks. The primary endpoint was brainstem-evoked response (BSER) audiometry improvement between baseline and 6 month follow-up, or for those with normal hearing at baseline, preservation of normal hearing at 6 months. Secondary endpoints included laboratory and clinical improvement, rate of growth and death.

At the outset, it should be recognized that the loss to follow-up rate was high and, therefore, denominators for each parameter vary. However, rigorous evaluation of drops indicated lack of bias in analyses. Twenty-one (84%) of 25 infants who were treated with ganciclovir and completed the study had hearing improvement or continued normal hearing at 6 months, compared with 10 (59%) of 17 patients in the group who received no treatment ($P=0.06$). At 6 month follow-up, none (0/25) of the ganciclovir recipients had hearing deterioration, while 7 (41%) of control patients did ($P<0.01$). Forty-three patients were followed and had BSER audiometry at 1 year of age or greater. Of these, 5 of 24 (21%) who had received ganciclovir had hearing deterioration in the best ear, compared with 13 of 19 (68%) of those in the control group ($P<0.01$).

Secondary outcomes showed significant short-term improvements in weight gain and head circumference in patients who were treated compared with controls. The treated group also had more rapid resolution of their liver function abnormalities. Patients who were treated and those who were not showed similar rates of resolution of hepatosplenomegaly and CMV retinitis.

The primary toxicity of ganciclovir, as shown in the previous study, was neutropenia. Twenty-nine (63%) of 46 patients who received ganciclovir developed moderate-to-severe neutropenia, compared with 21% of patients in the control group ($P<0.01$). Of these 29, 14 (48%) required dosage adjustments and 4 (14%) had the drug permanently discontinued.

This study demonstrates that 6 weeks of intravenous ganciclovir therapy prevents best-ear hearing deterioration during early childhood in patients with symptomatic congenital CMV affecting the CNS. However, the use of ganciclovir should be limited to those children with symptomatic disease, since the medication is mutagenic, teratogenic and carcinogenic.⁶³

Ganciclovir shows great promise for prevention of poor outcomes from congenital CMV infection, but is difficult to administer because of the requirement of an intravenous infusion. Thus, the administration of valganciclovir, the oral prodrug of ganciclovir, in the treatment of neonates with congenital CMV disease is being explored. Kimberlin and colleagues^{64,65} evaluated 24 neonates receiving 6 weeks of therapy with either intravenous ganciclovir or oral valganciclovir. The aim of the study was to assess the population pharmacokinetics of a pharmaceutical-grade oral valganciclovir solution to identify a dose that provided

Review

ganciclovir exposure comparable to the administration of intravenous ganciclovir in neonates with symptomatic congenital CMV disease. The study found that a 6 mg/kg intravenous ganciclovir dose and 16 mg/kg oral valganciclovir provide similar systemic exposures to ganciclovir. In addition, the pharmacodynamic analyses showed a median decrease in viral load of 0.7 log viral DNA copies/mL, in patients overall. Those who had the highest viral loads (>6 log viral DNA copies/mL) experienced greater decline in the viral load than those with lower baseline viral loads. Toxicity of valganciclovir is similar to that of ganciclovir with 38% of subjects developing moderate or severe neutropenia. Though results using pharmaceutical-grade valganciclovir cannot be extrapolated to pharmacy-generated formulations, these findings suggest that the oral valganciclovir solution may be a viable option for the treatment of symptomatic congenital CMV infection. Currently, the CASG is performing a controlled clinical trial of 6 weeks versus 6 months of valganciclovir therapy to determine whether a longer duration of treatment results in enhanced hearing and developmental benefits.

Unmet medical needs

Treatment of symptomatic congenital CMV improves audiological outcome. At this time, ganciclovir and its prodrug valganciclovir are the two medications that have been shown to be effective in the treatment of neonates with this common disease. Their use is limited by the potential for toxicity, namely induction of neutropenia, which can be particularly dangerous in neonates who are potentially more susceptible than uninfected or asymptomatic counterparts because of prematurity, residence in intensive care units and, in the case of ganciclovir, the risks of indwelling catheters for drug infusion. Maribavir, a benzimidazole L-riboside whose mechanism of activity has been mapped to the viral protein products of UL97 and UL27, may provide a new option for the treatment of congenital CMV disease.⁶⁷ Because maribavir does not need to be phosphorylated by UL97 kinase, as does ganciclovir, it has potential to be useful in the treatment of ganciclovir-resistant strains of CMV. Maribavir has undergone several Phase I and II studies in haematopoietic stem cell transplant recipients and, unlike ganciclovir, is not associated with nephrotoxicity or haematological toxicities. Phase III trials have begun in adult patients who have received stem cell and solid organ transplants. Maribavir may provide an alternative with less toxicity than existing medications in the treatment of congenital CMV infection.

Ultimately, CMV-associated hearing loss occurs in the vastly larger numbers of asymptotically infected babies than in the symptomatic group. However, antiviral drugs with improved toxicity profiles are required in order to justify the risk versus benefit of treatment. In addition to maribavir, other medications with CMV activity and favourable safety profiles are needed in order to achieve maximum therapeutic benefit in this potentially devastating disease.

Conceivably, new medications with alternative mechanisms of action may well lead to combination therapies as are employed in the management of AIDS.

Conclusions

Congenital CMV infection remains an important cause of neonatal morbidity and mortality and continues to greatly impact

the futures of both those who are symptomatic and asymptomatic at birth. Ganciclovir and valganciclovir provide effective reduction in hearing loss and improvement in development of those treated at birth. These are not perfect drugs, however, as their use is limited by their toxicities and their inability to cure patients of the disease. Future developments of treatments such as maribavir are beneficial, but more research and development can be done to continue to improve the lives of infants who are congenitally infected with CMV.

Funding

Work reported in this article was supported by a grant from the National Institutes of Health (NO-1-AI-30025) and the state of Alabama.

Transparency declarations

L. N. and D. K. have no declaration to report. R. W. is on the Board of Directors of Gilead Sciences and a consultant to Chimerix.

References

1. Stagno S, Britt W. Cytomegalovirus infections. In: Remington J, Klein J, Wilson C *et al.*, eds. *Infectious Diseases of the Fetus and Newborn Infant, Sixth Edition*. Philadelphia: Elsevier, 2006; 739–81.
2. Ribbert H. Über protozoenartigen Zellen in der Niere eines syphilitischen Neugeborenen und in der Parotis von Kindern. *Zentralbl Allg Pathol* 1904; **15**: 945–8.
3. Lowenstein C. Über protozoenartigen Gebilden in den Organen von Dindern. *Zentralbl Allg Pathol* 1907; **18**: 513–8.
4. Sinzger C, Grefte A, Plachter B *et al.* Fibroblasts, epithelial cells, endothelial cells and smooth muscle cells are major targets of human cytomegalovirus infection in lung and gastrointestinal tissues. *J Gen Virol* 1995; **76**: 741–50.
5. Plachter B, Sinzger C, Jahn G. Cell types involved in replication and distribution of human cytomegalovirus. *Adv Virus Res* 1996; **46**: 195–261.
6. Sinzger C, Jahn G. Human cytomegalovirus cell tropism and pathogenesis. *Intervirology* 1996; **39**: 302–19.
7. Halwachs-Baumann G, Wilders-Truschnig M, Desoye G *et al.* Human trophoblast cells are permissive to the complete replicative cycle of human cytomegalovirus. *J Virol* 1998; **72**: 7598–602.
8. Hemmings DG, Kilani R, Nykiforuk C *et al.* Permissive cytomegalovirus infection of primary villous term and first trimester trophoblasts. *J Virol* 1998; **72**: 4970–9.
9. Fisher S, Genbacev O, Maidji E *et al.* Human cytomegalovirus infection of placental cytotrophoblasts *in vitro* and *in utero*: implications for transmission and pathogenesis. *J Virol* 2000; **74**: 6808–20.
10. Hahn G, Baldanti F, Gallina A *et al.* Human cytomegalovirus UL131-128 genes are indispensable for virus growth in endothelial cells and virus transfer to leucocytes. *J Virol* 2004; **78**: 10023–33.
11. Weller TH. The cytomegaloviruses: ubiquitous agents with protean clinical manifestations. *N Engl J Med* 1971; **285**: 203–14.
12. Krech U, Jung M, Jung F. *Cytomegalovirus Infections of Man*. Basel: Karger, 1971.
13. Gold E, Nankervis GA. Cytomegalovirus. In: Evans AS, ed. *Viral Infections of Humans: Epidemiology and Control*. New York: Plenum, 1982; 167–86.

Review

14. Staras SAS, Dollard SC, Radford KW *et al.* Seroprevalence of cytomegalovirus infection in the United States, 1988–1994. *Clin Infect Dis* 2006; **43**: 1143–51.
15. Stagno S, Pass RF, Cloud G *et al.* Primary cytomegalovirus infection in pregnancy: incidence, transmission to fetus and clinical outcome. *JAMA* 1986; **256**: 1904–8.
16. Hayes K, Danks DM, Gibas H *et al.* Cytomegalovirus in human milk. *N Engl J Med* 1972; **287**: 177–8.
17. Lang DJ, Krummer JF. Cytomegalovirus in semen: observations in selected populations. *J Infect Dis* 1975; **132**: 472–3.
18. Reynolds DW, Stagno S, Hosty TS *et al.* Maternal cytomegalovirus excretion and perinatal infection. *N Engl J Med* 1973; **289**: 1–5.
19. Stagno S, Reynolds DE, Pass RF *et al.* Breast milk and the risk of cytomegalovirus infection. *N Engl J Med* 1980; **302**: 1073–6.
20. Bowden RA. Cytomegalovirus infection in transplant patients: methods of prevention of primary cytomegalovirus. *Transplant Proc* 1991; **23**: 136–8.
21. Taber LH, Frank AL, Yow MD *et al.* Acquisition of cytomegaloviral infections in families with young children: a serological study. *J Infect Dis* 1985; **151**: 948–52.
22. Schopfer K, Lauber E, Krech U. Congenital cytomegalovirus infection in newborn infants of mothers infected before pregnancy. *Arch Dis Child* 1978; **53**: 536–9.
23. Stagno S, Reynolds DW, Huang ES *et al.* Congenital cytomegalovirus infection: occurrence in an immune population. *N Engl J Med* 1977; **296**: 1254–8.
24. Boppana SB, Rivera LB, Fowler KB *et al.* Intrauterine transmission of cytomegalovirus to infants of women with preconceptual immunity. *N Engl J Med* 2001; **344**: 1366–71.
25. Stagno S, Pass RF, Dworsky ME *et al.* Maternal cytomegalovirus infection and perinatal transmission. *Clin Obstet Gynecol* 1982; **25**: 563–76.
26. Pass RF, August AM, Dworsky M *et al.* Cytomegalovirus infection in a day care center. *N Engl J Med* 1982; **307**: 477–9.
27. Adler SP, Wilson MS, Lawrence LT. Cytomegalovirus transmission among children attending a day care center. *Pediatr Res* 1985; **19**: 285A (abstract).
28. Murph JR, Bale JF, Perlman S *et al.* The prevalence of cytomegalovirus infection in a Midwest day care center. *Pediatr Res* 1985; **19**: 205S (abstract).
29. Adler SP. The molecular epidemiology of cytomegalovirus transmission among children attending a day care center. *J Infect Dis* 1985; **152**: 760–8.
30. Hutto C, Ricks R, Garvie M *et al.* Epidemiology of cytomegalovirus infections in young children: day care vs. home care. *Pediatr Infect Dis* 1985; **4**: 149–52.
31. Pass RF, Little EA, Stagno S *et al.* Young children as a probable source of maternal and congenital cytomegalovirus infection. *N Engl J Med* 1987; **316**: 1366–70.
32. Hutto C, Little A, Ricks R *et al.* Isolation of cytomegalovirus from toys and hands in a day care center. *J Infect Dis* 1986; **154**: 527–30.
33. Faix RG. Survival of cytomegalovirus on environmental surfaces. *J Pediatr* 1985; **106**: 649–52.
34. Stagno S. Breastfeeding and the transmission of cytomegalovirus infections. *Ital J Pediatr* 2002; **28**: 275–80.
35. Kreel I, Zaroff LI, Canter JW *et al.* A syndrome following total body perfusion. *Surg Gynecol Obstet* 1960; **111**: 317–21.
36. Seaman AJ, Starr A. Febrile postcardiotomy lymphocytic splenomegaly: a new entity. *Ann Surg* 1962; **156**: 956–60.
37. Armstrong JA, Tarr GC, Youngblood LA *et al.* Cytomegalovirus infection in children undergoing open-heart surgery. *Yale J Biol Med* 1976; **49**: 83–91.
38. Prince AM, Szmuness W, Millian SJ *et al.* A serologic study of cytomegalovirus infections associated with blood transfusions. *N Engl J Med* 1971; **284**: 1125–31.
39. Stevens DP, Barker LF, Ketcham AS *et al.* Asymptomatic cytomegalovirus infection following blood transfusion in tumor surgery. *JAMA* 1970; **211**: 1341–4.
40. Kaariainen L, Klemola E, Paloheimo J. Rise of cytomegalovirus antibodies in an infectious mononucleosis-like syndrome after transfusion. *BMJ* 1966; **1**: 1270–2.
41. Neiman PE, Reeves W, Ray G *et al.* A prospective analysis of interstitial pneumonia and opportunistic viral infection among recipients of allogeneic bone marrow grafts. *J Infect Dis* 1977; **136**: 754–67.
42. Winston DJ, Gale RP, Meyer DV *et al.* Infectious complications of human bone marrow transplantation. *Medicine (Baltimore)* 1979; **58**: 1–31.
43. Meyers JD, Flournoy N, Thomas ED. Nonbacterial pneumonia after allogeneic marrow transplantation: a review of ten years' experience. *Rev Infect Dis* 1982; **4**: 1119–32.
44. Fowler KB, McCollister FP, Dahle AJ *et al.* Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus infection. *J Pediatr* 1997; **130**: 624–30.
45. Harris S, Ahlfors K, Ivarsson S *et al.* Congenital cytomegalovirus infection and sensorineural hearing loss. *Ear Hear* 1984; **5**: 352–5.
46. Williamson WD, Percy AK, Yow MD *et al.* Asymptomatic congenital cytomegalovirus infection. Audiologic, neuroradiologic and neurodevelopmental abnormalities during the first year. *Am J Dis Child* 1990; **144**: 1365–8.
47. Hicks T, Fowler K, Richardson M *et al.* Congenital cytomegalovirus infection and neonatal auditory screening. *J Pediatr* 1993; **123**: 779–82.
48. Boppana SB, Pass RF, Britt WJ *et al.* Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. *Pediatr Infect Dis J* 1992; **11**: 93–9.
49. Weller TH, Hanshaw JB. Virologic and clinical observations on cytomegalic inclusion disease. *N Engl J Med* 1962; **266**: 1233–44.
50. Stagno S, Pass RF, Dworsky ME *et al.* Congenital and perinatal cytomegalovirus infections. *Semin Perinatol* 1983; **7**: 31–42.
51. Hanshaw JB, Dudgeon JA, eds. *Viral Diseases of the Fetus and Newborn*. Philadelphia: WB Saunders, 1978.
52. Osborn JE, Shahidi NT. Thrombocytopenia in murine cytomegalovirus infection. *J Lab Clin Med* 1973; **81**: 53–63.
53. Anderson KS, Amos CS, Boppana S *et al.* Ocular abnormalities in congenital cytomegalovirus infection. *J Am Optom Assoc* 1996; **67**: 273–8.
54. Stagno S, Reynolds DW, Amos CS *et al.* Auditory and visual defects resulting from symptomatic and subclinical congenital cytomegaloviral and toxoplasma infections. *Pediatrics* 1977; **59**: 669–78.
55. Boppana SB, Fowler KB, Vaid Y *et al.* Neuroradiographic findings in the newborn period and long-term outcome in children with symptomatic congenital cytomegalovirus infection. *Pediatrics* 1997; **99**: 404–14.
56. Dahle AJ, McCollister FP, Stagno S *et al.* Progressive hearing impairment in children with congenital cytomegalovirus infection. *J Speech Hear Disord* 1979; **44**: 220–9.
57. Dahle AJ, Fowler KB, Wright JD *et al.* Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus. *J Am Acad Audiol* 2000; **11**: 283–90.
58. Bale JF, Bray PF, Bell WE. Neuroradiographic abnormalities in congenital cytomegalovirus infection. *Pediatr Neurol* 1985; **1**: 42–7.
59. Perlman JM, Argyle C. Lethal cytomegalovirus infection in preterm infants: clinical, radiological, and neuropathological findings. *Ann Neurol* 1992; **31**: 64–8.

60. Boesch C, Issakainen J, Kewitz G *et al.* Magnetic resonance imaging of the brain in congenital cytomegalovirus infection. *Pediatr Radiol* 1989; **19**: 91–3.
61. Whitley RJ, Cloud G, Gruber W *et al.* Ganciclovir treatment of symptomatic congenital cytomegalovirus infection: results of a phase II study. *J Infect Dis* 1997; **53**: 1080–6.
62. Kimberlin DW, Lin CY, Sanchez PJ *et al.* Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: a randomized, controlled trial. *J Pediatr* 2003; **143**: 16–25.
63. American Academy of Pediatrics. Cytomegalovirus infection. In: Pickering LK, ed. *2006 Red Book: Report of the Committee on Infectious Disease, Twenty-seventh Edition*. Elk Grove Village, IL: American Academy of Pediatrics, 2006; 273–7.
64. Acosta EP, Brundage RC, King JR *et al.* Ganciclovir population pharmacokinetics in neonates following intravenous administration of ganciclovir and oral administration of a liquid valganciclovir formulation. *Clin Pharmacol Ther* 2007; **81**: 867–72.
65. Kimberlin DW, Acosta EP, Sanchez PJ *et al.* Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital cytomegalovirus disease. *J Infect Dis* 2008; **197**: 836–45.
66. Trofe J, Pote L, Wade E *et al.* Maribavir: a novel antiviral agent with activity against cytomegalovirus. *Ann Pharmacother* 2008; **42**: 1447–57.
67. Mocarski ES, Shenk T, Pass RF. Cytomegalovirus. In: Fields BN, Knipe DM, Howley PM *et al.* eds. *Virology* 5th edn. Philadelphia: Lippincott Williams and Wilkins, 2007; 2701–72.