Conference on Congenital CMV Infection

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Opening lecture:
The burden of congenital CMV disease
The Burden of Congenital CMV Infection

MJ Cannon

Centers for Disease Control and Prevention, (CDC), Atlanta, GA, USA

In many countries congenital cytomegalovirus (CMV) is the most common congenital infection, causing permanent disabilities such as hearing or vision loss, or mental retardation. More children are affected by serious CMV-related disabilities than by several better-known childhood maladies, including Down syndrome, fetal alcohol syndrome, and spina bifida. This presentation will catalog the health and economic burden created by congenital CMV infection, and highlight the urgent need for interventions that can reduce the substantial burden of this often overlooked disease.
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Pathogenesis (Session A)
Congenital CMV infection, impaired placental development and compensation after immunotherapy

L Pereira, E Maidji, T Tabata, S McDonagh

Cell and Tissue Biology Department, University of California San Francisco, San Francisco, CA, USA

CMV is the leading cause of congenital viral infection with an incidence in the United States of 1–3% of live births. Primary maternal CMV infection during gestation poses a 40% risk of intrauterine transmission in contrast to recurrent infection. Intrauterine growth restriction (IUGR), associated with congenital infection, and fetal loss without virus transmission originate in placental pathology (Benirschke, 2000).

Studies from our group revealed that CMV spreads from sites of infection in uterine arteries to invasive cytotrophoblasts, then to placental villi floating in maternal blood (Fisher, 2000; Maidji, 2002; Pereira, 2003). IgG with low neutralizing titer enable virus replication in villus cytotrophoblasts, then infection spreads to stromal fibroblasts and the fetal vasculature (McDonagh, 2006). Infected cytotrophoblasts impair differentiation and invasiveness in vitro (Fisher, 2000; Yamamoto-Tabata, 2004; Tabata, 2006). In early gestation, the neonatal Fc receptor for IgG transcytoses complexes with CMV virions that replicate in cytotrophoblast progenitor cells in the adjacent placenta (Maidji, 2006). Women with strong humoral immunity contain neutralizing IgG and virion gB in syncytiotrophoblasts without infection.

Women with primary CMV infection, treated at midgestation with intravenous hyperimmune globulin (HIG) with very high-avidity to CMV gB, significantly reduce symptomatic congenital disease as compared with untreated women (Nigro, 2006). We engaged in a collaboration to determine whether placental morphological changes could explain the efficacy of intravenous HIG. Histological analysis of biopsy specimens from infected placentas showed many large fibrinoids developed on floating villi that lacked cell structure and fetal blood vessels. We found a high level of syncytial knotting, associated with hypoxia and fetal IUGR in pregnancy disorders. Leukocyte infiltration, calcification and necrosis were detected in untreated infected placentas. Surprisingly, HIG-treated placentas developed small vascularized villi over the surface, suggesting compensation following resolution of infection. Underlying molecular mechanisms of placental impairment from viral pathogenesis, indirect damage from hypoxia, and villus development following HIG treatment will be discussed.
Model systems for studying congenital cytomegalovirus infection

MR Schleiss

_University of Minnesota Medical School Center for Infectious Diseases and Microbiology_
_Translational Research Minneapolis, MN, USA_

A vaccine against congenital CMV infection is a major public health priority, but no licensed vaccine is yet available. Therefore, the study of vaccines in animal model systems is of value in defining effective strategies for interrupting transmission and preventing disease in newborns. Among the small animal models of congenital CMV transmission, the guinea-pig CMV (GPCMV) is uniquely useful, because of the ability of the virus to cross the placenta and infect the fetus in utero. Recent characterization of the GPCMV has facilitated evaluation of vaccines in this animal model, data that in turn may be useful in informing clinical trial design in humans. The glycoprotein B (gB) homolog is a major target of neutralizing antibody response in guinea pigs infected with GPCMV, and expression of gB, both as a DNA vaccine as well as in soluble form as a purified protein subunit, has enabled vaccine studies. The gB protein subunit vaccine is highly effective at prevention vertical transmission and fetal disease. The magnitude of protection is dependent upon the adjuvant chosen for vaccination, and it is likely that adjuvant composition will be important for future clinical trials. Interestingly, fetal protection against disease does not depend upon induction of ‘sterilizing immunity’, but rather sufficient neutralizing response to reduce the magnitude of maternal DNAemia below levels that threaten fetal health. The role of other glycoproteins in the neutralizing antibody response is being increasingly understood, in particular the homologs of UL100 (gM), UL73 (gN), and a splice variant of UL73. This information is being applied to transmission studies in the guinea-pig model, to attempt to broaden the diversity of the neutralizing response to vaccines. Finally, the role of cell-mediated immunity to the GPCMV and its role in protection of the maternal-fetal unit, is being increasingly clarified. The GPCMV homolog of the UL83 protein, GP83, is a target of CD4+ and CD8+ responses in the setting of GPCMV infection, and expression of GP83 in the context of a subunit vaccine has been explored in the guinea-pig model. These results indicate that GP83 vaccine can reduce maternal DNAemia and improve fetal outcomes following virus challenge during pregnancy. These studies in the guinea-pig model will be put into context with other animal model data on CMV vaccines, and future directions in animal model evaluation of protective immunity for the fetus will be discussed.
ORAL COMMUNICATIONS
A-10. Maternal viral excretion and neutralizing antibodies in congenital
cytomegalovirus (CMV) infection in a highly seroimmune population

AY Yamamoto, MM Mussi-Pinhata, VM Wagatsuma, NR Barbieri, G Duarte

Department of Pediatrics, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão
Preto, São Paulo, Brazil

Background. Little is known about virologic and immunologic events of maternal CMV infection in
highly seroimmune populations.

Objectives. To determine CMV excretion rates in transmitter (T) and non-transmitter mothers (NT),
comparing serum CMV neutralizing (NTR) antibody titers around 13 weeks gestation and at delivery.

Methods. Fifty eight mothers of 50 infants identified as congenitally infected among 4439
neonates (incidence=1.1%) were enrolled. Congenital CMV infection was confirmed by DNA PCR
detection and viral isolation in urine and saliva samples obtained within 2 weeks of birth. In a case-
control study, for each index case, 3 seropositive mothers (144) of non-infected infants were select-
ed. Breast milk, urine, saliva and blood were obtained from T and NT mothers after delivery (medi-
an=3 days) and processed by a qualitative DNA-PCR. Positive breast milk samples were quantified
by a real-time PCR. Virus NTR activity was determined with a microneutralization assay.

Results. Overall, CMV was more frequently detected in T than NT mothers (41/48 (85%) vs. 39/144
(27%) p<0.0001; OR=15.77) like virus excretion in > 1 site was (19.5% vs. 2.6%; p=0.001). The shed-
ing rate for breast milk was significantly higher in T (38/44; 86%) than in NT mothers (39/143; 27%)
(p<0.0001; OR=16.89) as it was in urine (9/48; 19% vs. 0/140; 0%; p<0.0001). However, viral excretion
was infrequent in saliva (2/48; 4% vs. 1/142; 1%; p=0.569). No CMV DNA was detected in
blood. Breast milk viral DNA load was higher in T than in NT mothers (GMT= 2.37x102 (2.6x10-2.31x103)
vs. 5.1x10 (1.2x10 - 3.43x102) copies/µL, p=0.004). All 26 T and 67 NT mothers who had
available serum specimens at the beginning of pregnancy were CMV seropositive. By then, lower
NTR titers were found in T mothers (GMT= 87.5(4 to 128) vs. 142(32 to 256); p=0.02) while no dif-
fERENCE was detected at delivery (GMT= 256(32-512) vs. 256(16-1024); p=0.16. Though, higher rises
in NTR antibody titers from the first trimester to delivery were observed in T mothers (p=0.019).

Conclusions. Particularly in colostrum, CMV excretion close to delivery is common in mothers of
infants with congenital infection. High virolactia correlates with transmission of CMV to the fetus
and can be a marker for viral replication in maternal less accessible sites. The presence of low neu-
tralizing CMV antibody titers at the beginning of gestation may be a risk factor for intrauterine viral
transmission.
A-49. Human cytomegalovirus causes migration disturbances in human neural precursor cell cultures

N Wolmer, C Söderberg-Nauclér, J Odeberg

Department of Medicine, Center for Molecular Medicine, Karolinska Institute, Stockholm, Sweden

The clinical evidence of the effect of human cytomegalovirus (HCMV) on the development of the central nervous system has been known for more than 50 years, but at present the mechanisms behind these disturbances are unknown. Animal experiments have revealed that murine CMV can affect several important cellular functions of the neural stem cells, such as impaired growth, differentiation and migration. Since CMV is species specific, we want to further analyse whether HCMV also exhibit negative effects on the cellular functions of human neural stem cells, which may explain the disturbances of the brain development observed in the clinic.

In recent years we have developed a system for culturing human foetal neural precursor cells from the sub ventrical zone of the forebrain, were HCMV permissive stem and precursor cells are located. The cells are grown in vitro as neurospheres, keeping their immature phenotype (expressing markers for immature cells, such as nestin and CD133) for several passages. Using this cell culture system we have assessed different aspects of the neuronal development process such as differentiation, proliferation and apoptotic patterns. We have recently shown that both differentiation of neural precursor cells (NPCs) into neurons and astrocytes is impaired in infected cultures.

Since earlier work has shown that migratory disturbances also contribute to the symptoms observed after congenital HCMV infection, we further investigated migration properties of infected and uninfected NPCs in a transwell system as well as FACS analysis of receptors known to be important for migration. Interestingly, our preliminary findings demonstrated decreased migration abilities in infected cells accompanied by down regulation of the expression of platelet derived growth factor receptor α (PDGFRα) and the chemokine receptor CXCR4 on the cell surface 7-10 days post infection. These findings indicate a mechanism where HCMV may regulate the cell surface expression of receptors and thereby influencing biological functions of the NPCs. This would be in accordance with findings of the effect of HCMV in other cell types, such as dendritic and endothelial cells. Further studies of the mechanism behind the interaction between HCMV and its immature host cell may lead us to new conclusions on cell signalling and regulation of biological networks as well as stem cell behaviour.
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Diagnosis in the mother, fetus and newborn (Session B)
Cytomegalovirus (CMV) is the leading cause of congenital infection in the developed countries, occurring in 0.3–2% of all live births.

Mother-to-child transmission is mainly the result of primary maternal CMV infection which carries a risk of transmission varying from 24 to 75% (mean value 40%). Cases of CMV transmission due to nonprimary infection have been reported in 1-2.2% of cases, i.e. at a much lower rate than those resulting from primary infection.

Within the last ten years, European laboratories have made significant progress in solving diagnostic problems linked to infection in pregnancy. With the advances in CMV serology, the presence of anti CMV IgM detected by a screening test such as EIA, can be confirmed by blot, identifying pregnant women undergoing an active or recent infection. Furthermore, primary infections that were proven if a seroconversion was observed or suspected in the presence of IgM, can now be readily diagnosed by disclosing the presence of anti-CMV low/moderate avidity in IgM-positive mothers, greatly reducing the number of women who should be considered at risk of transmitting the virus.

In several Italian regions, pregnant women are tested for CMV during the first trimester of pregnancy. The screening is performed with commercially available kits for both anti CMV IgG and IgM. Seronegative women are rechecked at the end of the fourth month of pregnancy. Women with test results showing seroconversion or IgM positivity are sent to a referral center for further investigations.

A cohort of 2477 pregnant women considered at risk of transmitting the virus were followed in a longitudinal study at the University of Bologna. Women were identified as part of routine CMV screening in several Italian regions and were IgM positive for CMV.

The majority of the pregnant women (55.1%) had no active infection and were classified as uninfected or not actively infected. Primary CMV infection was documented in 28.1% of cases.

However, only 30% of primarily infected mothers transmit the virus, opening a major diagnostic problem that can be solved by in utero diagnosis.

In utero diagnosis carried out by virus isolation and by qualitative and quantitative PCR in amniotic fluid of primarily infected mothers taken at 21–22 weeks gestation and at least 6–8 weeks after seroconversion can identify fetuses that are not at risk of congenital infection, and fetuses with a low viral load which should not be considered at risk of developing a symptomatic congenital infection. These results can help the physician to counsel infected mothers thus allowing many more mothers to continue their pregnancy with confidence.

Conference on Congenital Cytomegalovirus Infection

Advances in the diagnosis of materno–fetal CMV infection

T Lazzarotto

U.O. di Microbiologia, Policlinico S. Orsola Malpighi, University of Bologna, Italy
Diagnosis and management of fetal infection. A perinatologist perspective

Y Ville

Service de Gynécologie Obstétrique, Hôpital de Poissy-St-Germain, Université of Paris, Poissy, France

Human cytomegalovirus (CMV) is the main cause of mental retardation and sensorineural hearing loss related to congenital infections. Justification of systematic screening for fetal CMV infection is still controversial and is not recommended in most developed countries. This is mainly justified by the paucity of antenatal prognostic factors and the lack of established intrauterine treatment when fetal infection has been diagnosed. Ultrasound screening is widely used in the second trimester of pregnancy and perinatologists should be aware of the non-specific ultrasound features of fetal infection. When faced with a suspicious ultrasound, maternal serology may already show isolated IgG and IgM may have already disappeared, and the sue of a booking sample when available and that of IgG avidity may prove critical. The diagnostic work-up should be strict and allow for a 6-week interval from seroconversion to amniocentesis. Prognostic factors in maternal and fetal blood are still ill-defined and viral load in the amniotic fluid is controversial. Serial fetal assessment may include, serial targeted ultrasound, MRI of the fetal brain and fetal blood sample. At least two pathways have recently been opened for fetal therapy including hyper-immune immunoglobulins and antiviral drugs, mainly valaciclovir.
ORAL COMMUNICATIONS
B-01. Under-reporting of infant mortality caused by congenital cytomegalovirus infection

B Camp, B Sirotkin, MJ Cannon

Centers for Disease Control and Prevention, Atlanta, GA, USA

Background. Cytomegalovirus (CMV) is the leading congenital viral infection in the U.S., causing hearing and vision loss, mental retardation and death in affected newborns. Infection presents nonspecifically, which may complicate diagnosis and may cause the number of CMV-related infant deaths to be under-reported. To assess this possibility, we compared the number of infant deaths reported on death certificates to the National Center for Health Statistics (NCHS) with the best available estimates of infant deaths reported in the literature for CMV, congenital herpes simplex virus (HSV), anencephaly, Down syndrome and spina bifida.

Methods. We used International Classification of Diseases–10 (ICD–10) diagnostic codes to search 1999–2001 NCHS death tapes for multiple cause infant deaths. We performed literature searches to find prevalence and infant mortality rates for each disease; preference was given to studies using screening and active surveillance methods. The estimated annual number of infant deaths was calculated using prevalence and mortality rates, assuming 4 million live births per year.

Results. In the U.S., the average annual number of infant death certificates listing each disease as the underlying or contributing cause of death, and the annual number of infant deaths as determined from best literature estimates, respectively, are 28 and 439 for CMV; 26 and 120 for HSV; 227 and 420 for Down syndrome; 49 and 65 for spina bifida; 312 and 408 for anencephaly.

Conclusions. The average annual number of multiple cause infant death certificates listing congenital CMV and HSV was considerably less than the estimated number of annual infant deaths from literature sources. In contrast, the death certificate numbers for anencephaly, Down syndrome, and spina bifida were more similar to the literature estimates. While imprecise, these figures suggest that death certificates may underestimate the deaths caused by diseases that present nonspecifically, such as CMV. Increased awareness and newborn screening for congenital CMV may lead to more accurate diagnosis, documentation, and assessment of infant mortality. Furthermore, increased newborn screening may help identify both asymptomatic and misdiagnosed symptomatic cases of congenital CMV. Future appraisals of newborn CMV screening programs should take into account the benefit of detecting misdiagnosed symptomatic cases, since cost-benefit analyses might only consider the benefit of detecting asymptomatic cases.
B-23. Epidemiology of materno–fetal CMV infections in France

I Parent du Châtelet,1 L Grangeot-Keros,2 A Leblond,1 Y Le Strat,1 P Lebon,2 F Freymuth,1 F Jacquemard,4 Y Aujard,1 C Six,1 D Lévy-Bruhl1

1National Institute for Health Surveillance, Saint–Maurice; 2A. Becleere Hospital, Clamart; 3Saint-Vincent de Paul Hospital, Paris; 4Clemenceau Hospital, Caen; 5Institut de Puericulture, Paris; 6R. Debre Hospital, Paris

Background/Objectives. The disease burden of maternal and congenital CMV infection is imperfectly known in France. From November 2004 to January 2005, a national prospective laboratory based study was undertaken to estimate the incidence of CMV infections acquired by fetuses and diagnosed during pregnancy or at birth and to describe clinical or biological features that led the clinicians to request CMV testing.

Methods. In 2003, a preliminary study showed that IgG avidity index testing is performed in 98% of pregnant women with positive CMV IgM. All French laboratories performing IgG avidity and/or virus detection were asked to provide aggregated data on results indicating a CMV infection in pregnant women, fetuses or neonates during the study period.

Case-based notifications were done by a sub-sample of laboratories and demographic and clinical data were secondarily collected from clinicians.

For annual incidence estimation, we took into account the number of cases newly diagnosed and captured by the sub-sample of laboratories and the proportion of these cases among the overall number of tests performed in France.

Results. Seventy four congenital infections were identified through the laboratories sub-sample. Twenty-one cases were symptomatic (8 confirmed by fetal pathologic examination and 13 with symptoms present at birth). Ultrasound abnormalities were detected during pregnancy in 14 cases (67%). Data on maternal infections, diagnosis practices (including serology testing and prenatal diagnosis) and pregnancy outcomes were also analysed.

The annual incidence of congenital infections diagnosed during pregnancy or at birth was estimated at 277 cases [CI95%: 204-349]. Among them 30 [CI95%: 23-37] would lead to pregnancy terminations with pathologic examinations and 46 [CI95%:33-59] to symptomatic CMV infected newborns corresponding to an incidence rate of 5.9 per 100 000 live births [CI95%: 4.2-7.6].

Conclusions. The results are compatible with other estimations in France. They allow estimating that more than 50% of symptomatic CMV congenital infections that occur in France are diagnosed during pregnancy or at birth and that less than 15% of asymptomatic infected infants are also routinely detected according to the current level of serological testing. These data, with additional data on clinical follow up of infected newborns will be useful to complete the analysis of the relevance of systematic serological screening in pregnant women in France.
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Prognostic markers & Counselling (Session C)
Searching for prognostic markers of HCMV transmission and disease

MG Revello

Servizio di Virologia, IRCCS Policlinico San Matteo, Pavia, Italy

With no vaccine in sight for the near future and in the absence of screening programmes, congenital HCMV infection is likely to remain a significant public health problem for many years to come. In this gloomy scenario, the availability of reliable markers for the identification of: i) infected women at risk of transmission \textit{in utero}; and ii) infected fetuses/newborns at risk of developing HCMV disease or sequelae would be crucially important for both counselling and management of pregnant women diagnosed with primary HCMV infection as well as for monitoring of congenitally infected infants. Unfortunately, the identification of infected women who will transmit the infection is still more a challenge than an achievement. In fact, although recent data indicate that HCMV-specific cell-mediated immune response is significantly reduced in transmitter mothers, these results cannot be used on a prospective basis for individual counselling at the moment. On the other hand, advancements have been made in the definition of the prognostic value of viral load in amniotic fluid and fetal blood for the identification of infected fetuses, as well as of quantification of HCMV in blood at birth and during infancy as predictor of sequelae. Finally, the association of genetic polymorphisms of the virus with outcome of congenital infection has still to be defined.

In conclusion, since reliable prognostic markers of HCMV transmission/disease remain elusive, great caution must be exercised in the clinical use of available information, and much effort should be directed to the prevention of primary HCMV infection in the pregnant woman.
Ethical dimensions of the fetus as a patient

FA Chervenak,' LB McCullough

'Weill Medical College of Cornell University, 'Baylor College of Medicine

The goal of this presentation is to describe the ethical concept of as a patient and to identify its implications for the counselling of pregnant women about the management of pregnancy complicated by fetal anomalies, including those caused by CMV infection, for fetal research, and for patient autonomy.

The presentation will begin with an account of obstetric ethics, with a focus on the basic ethical principles of beneficence and respect for autonomy. The ethical concept of the fetus as a patient will be explicated in terms of the physician's beneficence-based and autonomy-based obligations to the fetal patient and the pregnant woman's beneficence-based obligations to the fetal patient. This explication of the ethical concept of the fetus as a patient will distinguish between independent moral status, which does not apply, and dependent moral status, which does apply.

Based on this ethical concept of the fetus as a patient, the topic of termination of pregnancy will be addressed. The ethical concept of the fetus as a patient requires the physician to engage in non-directive counselling of the pregnant woman when an anomaly is detected before viability: the physician should present all medically reasonable alternatives, including termination of pregnancy, should be presented to the pregnant woman and no recommendation should be made about which she should elect. This is her autonomous decision to make. The physician's individual conscience justifiably limits participation in termination of pregnancy, but not in the informed consent process.

After viability the options of aggressive management, third-trimester abortion, cephalocentesis, and non-aggressive management will be analyzed for their ethical justification and clinical application.

Counseling pregnant women about fetal research should be non-directive. The concept of equipoise and its application to initiating randomized clinical trials will be discussed.

The ethical concept of prenatal informed consent for sonogram (PICS) will be described as an autonomy-enhancing strategy.
ORAL COMMUNICATIONS
C-36. Non-virological markers of infection and damage in human cytomegalovirus-infected fetuses

B Tassis, MG Revello,* G Gerna,* M Zavattoni,* M Furione,* U Nicolini

Department of Obstetrics and Gynecology, Ospedale V. Buzzi, University of Milano;
*Department of Virology, IRCCS Policlinico San Matteo, Pavia, Italy

Objectives. To assess the presence of non-virological maternal and fetal parameters that correlate with maternal transmission and fetal damage of human cytomegalovirus (HCMV) infection.

Methods. Seventy-six women with documented primary HCMV infection underwent prenatal diagnosis from 18 to 26 weeks’ gestation. In addition to HCMV-specific assays, maternal and fetal blood samples were tested for white blood cell and lymphocyte count, percentage of CD3+, CD4+, CD8+, CD56+, HLA-DR-CD3+ T lymphocyte subpopulations and CD19+ B lymphocytes. Acid-base balance, haemoglobin concentration, platelets count, beta2-microglobulin and liver enzymes were also determined in the fetuses and compared to our own reference ranges.

Results. The 37 women (49%) who transmitted the infection had significantly lower white blood cell counts compared to those mother carrying fetuses without infection. Percentage of CD8+ and HLA-DR-CD3+ T lymphocytes were higher and percentage of CD4+ and the CD4+/CD8+ ratio lower in the infected compared to the non infected fetuses.

Moreover, infected fetuses showed lower platelets count and higher levels of GGT and beta2-microglobulin compared to non infected fetuses. Platelet count, ALT and beta2-microglobulin were the only parameters that differed between the 19 infected fetuses normal at follow-up and the 7 infected fetuses with clinical signs (3 false negative diagnosis and 8 termination of pregnancy were excluded). No difference occurred in maternal and fetal blood cell counts and lymphocyte subpopulations in these 2 groups of infected fetuses. Acid-base balance, haemoglobin and AST values were also similar.

Conclusions. The infected fetuses and their infected mothers mount a normal immunological response following HCMV infection and fetal damage does not seem to be related to increased or depressed immunological response. Fetal serum beta2-microglobulin achieved best specificity (94%) and best sensitivity (95%) in the prediction of fetal infection.
C–26. Are HCMV DNA loads in urine and blood of infected pregnant women pronostic markers of congenital infection in their newborns?

F Brancart, C Donner, B Vincart, F Gosselin, M–L Delforge, A Marchant, C Liesnard

Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium

Objective. To investigate the relationship between HCMV DNA load in blood and urine of women presenting a HCMV primary infection during pregnancy and the presence of a congenital infection in their newborns.

Methods. HCMV DNA load was determined in blood and urine of 102 women at risk for HCMV primary infection during pregnancy on the basis of their serologic results. Samples were taken at their first visit at the Fetal Medicine Unit and at follow-up visits when possible. The time elapsed between the first HCMV positive serology and the collection of blood and urine samples was calculated in days. HCMV DNA load was determined using an in–house real–time PCR on the pp150 gene. Extraction of blood and urine was done using the Qiagen DNA blood mini kit (Qiagen, Benelux). HCMV quantification was done using a commercial quantitated control (CMV AD169 quantitated DNA control, ABI, Columbia, MA). Fetal or newborn HCMV infection was determined using real–time PCR and viral culture on amniotic fluid sampled during pregnancy or on saliva and urines of newborns at birth or on organs of aborted fetuses.

Results. Among 102 pregnancies, 35 were infected. 29 urine and blood samples were collected among infected pregnancies and 81 urine and 60 blood samples among non infected pregnancies. HCMV was detected in 65.5% of urine sample and 37.9% of blood samples among infected pregnancies and in 46.9% of urine samples and 28.3% of blood samples among non infected pregnancies. There was no statistical differences between detection of HCMV in urine and blood in infected and non infected pregnancies. The time elapsed between the mother’s HCMV infection and samples collection was not statistically different between infected and non infected pregnancies. HCMV load in urine ranged from 2.03 to 5.5 log_{10}/mL. There was no statistical difference in HCMV load in urine between infected and non infected pregnancies. HCMV load in blood ranged from 1 copies to 5264 copies/10^8 cells. Blood HCMV load was higher in infected pregnancy than in non infected pregnancies (p=0.01), but no clear cut–off could be established because of the low number of evaluated blood samples.

Conclusions. We found no relationship between HCMV DNA load in urine of infected pregnant women and the presence of a congenital infection in their babies. HCMV DNA load in blood of infected pregnant women could be a risk marker for transmission of the virus to the fetus. Further studies should be conducted to confirm our results.
C-08. One year epidemiological study of CMV infection during pregnancy in a French hospital

O Picone,1 C Vauloup-Fellous,2 MV Senat,1 R Frydman,1 L Grangeot-Keros1

1Service de Gynécologie Obstétrique; 2Service de Microbiologie Hôpital A. Béclère, Clamart, France

Background/Objectives. Although CMV infection during pregnancy is quite frequent, epidemiological data of this infection in France are scarce. Screening of CMV infection during pregnancy is debated, and is not recommended in France. However, it is recommended to inform the patient about CMV infection during pregnancy. The goals of this study were i) to evaluate the frequency of women agreeing for screening after information on the consequences of CMV infection during pregnancy, ii) to collect epidemiological data on CMV infection and its consequences during pregnancy, in our center, in 2005.

Methods. Each pregnant woman followed in our centre is informed on CMV congenital infection. The possible consequences of a primary infection, and the lack of scientific data concerning the interest of screening are explained. Therefore, if the patient agrees, a serologic testing is performed around 12 and 32 weeks of gestation. If a seroconversion occurs, a close fetal ultrasound examination and amniocentesis are systematically proposed. If the first CMV serologic test is negative, hygienic informations are given to the mother and her husband. Moreover, in our center, a systematic screening of hearing loss is performed to all newborns.

Results. In 2005, CMV screening was proposed to 2099 patients. Only 95 refused (4.5%). 2004 patients were tested. The first serological test performed around 12 weeks of gestation showed that 1146 (57.2%) were IgG negative, and 858 (42.8%) were IgG positive. Among IgG positive patients, 67 were found IgM positive. IgG avidity was measured in these 67 cases, and primary infection was excluded (high IgG avidity) in 51 patients (76%). Overall, analysis of serum testing allowed detection of 4 seroconversions among initially seronegative women (4/1146, 0.35%) and 5 primary infection (IgG+, IgM+, low IgG avidity) among initially seropositive patients (5/858, 0.58%). 2 children out of 9 were born infected. None were symptomatic or demonstrated prenatal ultrasound features, and, up to now, none has developed any sequelae.

Conclusions. Our study demonstrated that: i) if clear information on CMV infection during pregnancy is given, patients very frequently agree for screening, ii) seroprevalence is similar to those reported in western Europe, iii) the rate of seroconversion seems low which encourages counselling. Concerning screening, if proposed, the best way to perform it, is still to define.
C-48. Impact of confirmatory tests and prenatal counseling on the rate of pregnancy termination among women with positive CMV-IgM titers

B Guerra, G Simonazzi, A Banfi, T Lazzarotto, A Farina, L Gabrielli, M Lanari, N Rizzo

Departments of Obstetrics and Gynecology, Clinical and Experimental Medicine, Sect. of Microbiology; St. Orsola Malpighi Hospital, University of Bologna, Italy; Department of Pediatrics and Neonatology, La Scaletta Hospital, Imola, Bologna, Italy

Objectives. To determine if diagnostic tests performed in a reference laboratory and the correct interpretation and communication of results by an expert physician to the patient can reduce the rate of unnecessary abortions among women with positive cytomegalovirus (CMV) immunoglobulin M antibody titers.

Study Design. This was a retrospective study of 1857 consecutive pregnant women with positive screening for IgM anti-CMV, in the first or second trimester of pregnancy, referred to our unit for further diagnostic evaluation. Patients with available follow-up were divided into two groups according to the results of confirmatory serologic testing: women with a CMV serologic profile suggestive of primary infection and hence at high risk of vertical transmission (group 1) and women with a CMV serologic profile consistent with nonprimary infection or past infection (group 2). The number of expected pregnancy terminations and the prevented fraction of abortions were calculated.

Results. Of 445 group 1 patients, 53 (11.9%) elected to terminate the pregnancy after being informed of the results of diagnostic tests; in contrast, only five (0.4%) women in group 2 underwent terminations (p<.001). At autopsy, 38 fetuses in group 1 proved infected. No information on fetal infection is available for pregnancies terminated in the first trimester (15 in group 1; five in group 2). We estimated that ≥196 (11.9%) of all patients in groups 1 and 2 (n=1650 patients) would have elected abortion on the basis of the positive result of screening for fetal CMV infection. After the results of confirmatory tests only 58 women (53 in group 1 and five in group 2) elected to terminate the pregnancy. Thus the number of abortions is presumed to have been decreased by 73% (p<.001).

Conclusions. The correct interpretation and communication of confirmatory test results by expert physicians to pregnant women with positive screening for IgM anti-CMV may significantly reduce the rate of unnecessary abortions.

References
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Immune response (Session D)
T cell development in human cytomegalovirus infection

LE Gamadia,1,2 EB Remmerswaal,1 IJ ten Berge2 RA van Lier1

1Laboratory for experimental immunology, 2renal transplant unit,
Academic Medical Center, AMC, the Netherlands

The correlates of protective immunity to disease inducing viruses in humans are complex and involve T cells, B cells and antibodies. In immunocompromised patients, normally non-pathogenic viruses can cause severe disease, whereas in healthy individuals these viruses are asymptomatic. In this presentation the differences in immune responses to CMV in asymptomatic and symptomatic CMV infection are discussed. Also the phenotype of CMV specific cells in latency and in reactivation is assessed as is the effect of the development of CMV-specific CD8+ T cells on the total T cell pool.
Cellular immunity to CMV during foetal life

A Marchant, M Tackoen, J Renneson, N Vanderheyde, C Liesnard, C Donner

Institute for Medical Immunology, Charleroi, and Hôpital Erasme, Brussels, Belgium

The susceptibility of foetuses to infection with CMV suggests ineffective cell-mediated immune response to the virus. In adults, CMV induces large oligoclonal expansions of CD4 and CD8 T lymphocytes expressing a phenotype of advanced differentiation. This phenotype is characterised by the loss of expression of two co-stimulatory molecules, CD28 and CD27. CMV-specific CD8 T lymphocytes produce anti-viral cytokines, including interferon (IFN)-γ, and express perforin-dependent cytolytic activity. CMV-specific CD4 T cells proliferate and produce IFN-γ. We demonstrated that congenital CMV infection induces a CD8 T cell response that has similar characteristics to that of adults with a late differentiation phenotype, anti-viral cytokine production and cytolytic activity. In contrast, foetal CD4 T lymphocytes display a defective response to CMV. Oligoclonal expansions of CD4 T cells can be detected in infected foetuses. A proportion of the CD4 T lymphocytes also express a late differentiation phenotype and produce perforin. In contrast, no cytokine or proliferative responses can be detected following in vitro restimulation with CMV antigens, suggesting that these cells are anergic. Data obtained in the mouse model of CMV infection suggest that the defective CD4 T lymphocyte response could play a role in the prolonged excretion of the virus observed in children infected in utero. The dissociation between the CD8 and CD4 T cell responses to congenital CMV infection could be related to a different effect of the virus on fetal as compared to adult dendritic cells.
ORAL COMMUNICATIONS
D-02. Development of human cytomegalovirus-specific T-cell-mediated immune response in pregnant women with primary infection

C Fornara, D Lilleri, MG Revello, G Gerna
Servizio di Virologia, IRCCS Policlinico San Matteo, Pavia, Italy

Objective. To investigate the development of human cytomegalovirus (HCMV)-specific T-cell mediated immune response in immunocompetent individuals undergoing primary HCMV infection.

Methods. 42 immunocompetent individuals (37 pregnant women) with ascertained primary HCMV infection were investigated. HCMV-specific lymphoproliferative response (LPR) and cytokine production were analysed during the first year after onset of infection. Proliferation of HCMV-specific CD4+ and CD8+ was detected by carboxyfluorescein succinimidyl ester-based flow cytometry assay, and a cell division index (CDI) was calculated. Cytokine flow cytometry was used to determine IFN-γ and IL-2 production by T-cells. CDI values <3 indicated a negative LPR and CDI values >15 indicated a full response (as detected in HCMV-seropositive control subjects with remote infection); CDI values between 3 and 15 revealed an initial response detected only during primary infection.

Results. CD4+ T-cell LPR developed earlier than CD8+ LPR (p=0.045). However, CDI values for both T-cell subpopulations were lower than in seropositive controls. A strong correlation between development of HCMV-specific LPR and virus disappearance from blood was observed. IFN-γ producing CD4+ and CD8+ T-cells were already observed during the first month after infection, while IL-2 producing T-cells were very infrequently detected in blood. CD4+ LPR developed later in pregnant women transmitting HCMV to the fetus compared to non-transmitting women.

Conclusions. During a primary HCMV infection, LPR and IL-2 production by circulating T-cells develops later as compared to IFN-γ production. CD4+ T-cell LPR develops earlier than CD8+ LPR, both persisting in lower magnitude as compared to control subjects with remote infection. Prospective studies aimed at understanding the correlation between immune response development and HCMV transmission to the fetus are warranted.
D-25. T lymphocyte response to congenital CMV infection

M Tackoen, W Burny, N Vanderheyde, S Lecomte,
C Liesnard, C Donner, A Marchant

Institute for Medical Immunology and Hôpital Erasme, Université Libre de Bruxelles, Belgium

Congenital CMV infection is associated with defective interferon (IFN)-γ and proliferative responses of CD4 T lymphocytes. This study was undertaken to evaluate whether foetal CD4 T lymphocytes are activated by CMV in utero and to determine the characteristics of these cells. Cord blood was collected from neonates born to mothers with primary CMV infection during pregnancy. In newborns with congenital infection (n=8) we detected a population of CD4 T lymphocytes (6,557±14,224%) expressing an advanced differentiation phenotype (CD27−/CD28−) which was not detectable in uninfected babies (n=18). The differentiated CD4 T cell population also expressed perforin. Stimulation of CMV seropositive adult peripheral blood mononuclear cells (PBMC) with a lysate of CMV-infected fibroblasts stimulated the production of IFN-γ and CD154 by CD4 T cells. In contrast, no significant responses could be detected in infected newborns. Similarly, no accumulation of the type 2 cytokines IL-4 and IL-13 mRNA was detected by quantitative PCR. No evidence for a regulatory T cell response was found as PBMC from infected newborns contained similar proportions of CD25-CD4 T cells and similar levels of IL-10 and FoxP3 mRNA. In addition, in vitro stimulation of purified CD4 T cells with autologous monocytes and CMV antigens failed to induce detectable cytokine or proliferative responses in infected newborns. These results indicate that CMV induces the differentiation of foetal CD4 T lymphocytes expressing perforin and suggest that these cells are anergic.
D-37. Functional virus-specific CD8+ T cells but defective CD4+ T cells in infants with congenital and postnatal CMV

AK Lidehäll, M-L Engman, F Sund, G Malm, I Lewensohn-Fuchs, U Ewald, TH Tötterman, O Korsgren, BM Eriksson

Div of Clinical Immunology, Uppsala, Sweden

Background. While primary CMV infections in adolescents and adults are brought into a latent state within months, children born with congenital CMV have been shown to excrete virus in saliva and urine for up to 10 years. Recent development of new immunological techniques has enabled characterization of the deficiency of the immune system causing this prolonged viral shedding. We here investigate CMV-specific T-cell function in children with congenital CMV.

Methods. Blood samples were drawn and analyzed from 13 children at 1.5-30 months of age (median: 5.5 months). For comparison, seven children with postnatally acquired CMV and seven adults with primary CMV were investigated. CMV-specific T-cell function was analyzed by in vitro stimulation of CD4+ (T helper) and CD8+ (T killer) cells, respectively, followed by intracellular cytokine staining.

Results. IFNγ production in CMV specific CD8+ cells was detected in all children with congenital and postnatal CMV regardless of age at analysis. CMV-specific CD4+ function was however impaired compared to adults with primary infection. Median IFNγ producing cells in adults during their first 6 months of primary infection was significantly higher than in children 0-6 months with postnatal or congenital CMV (p<0.001). In all seven adults a rapid up-regulation of CMV-specific CD4+ T cell function was observed within the first two months after appearance of symptoms. In children younger than 6 months, IFNγ production was above detection limits in only 2 of 6 children with congenital and in 0 of 6 with postnatal CMV.

Conclusions. Our results imply that CMV-specific CD4+ deficiency might contribute to slow clearance of CMV in children.
**Background and Objectives.** Congenital cytomegalovirus (CMV) infection remains a significant cause of morbidity and mortality in young children. Moreover, children may shed virus for prolonged periods following primary CMV infection. CMV-specific CD8⁺ T cells are associated with control of viral replication and protection from severe disease in adults. We previously showed that CMV-specific CD8⁺ T cell responses to CMV pp65 (UL83) and immediate early-1 (IE1) proteins were readily detectable, and their detection correlated temporally with resolution of viremia in children with congenital or postnatal primary CMV infection. We have further characterized the evolution of CMV epitope-specific CD8⁺ T cell responses over the course of CMV infection in these children.

**Methods.** Seven infants less than 6 months of age at study onset were evaluated. CD8⁺ T cell lines and overlapping peptides spanning the pp65 and IE1 proteins were incubated in ELISpot assays to map CMV epitopes and determine functional peptide avidity of detectable CMV epitope-specific responses. Chromium release assays were used for HLA restriction analysis of these responses.

**Results.** At the time of initial testing (1–6 months of age), CMV-specific CD8⁺ T cell responses were detected in all infants. Responses targeted 13 pp65 and 14 IE1 peptides, including epitopes not previously reported. The antigenic diversity of these responses remained stable or increased over time in all infants despite clearance of CMV viremia. Loss of epitope recognition was not observed. Epitopes defined in adults were recognized, but not by all infants with the appropriate HLA allele. Functional peptide avidity of CMV-specific responses varied between individual infants and peptides, but was highest for peptides reported as immunodominant in adults with latent CMV infection. In all infants, the avidity of responses to individual peptides remained stable or increased over time.

**Conclusions.** These data provide additional evidence that young infants can generate virus-specific T cell responses with a high degree of antigenic diversity and avidity for cognate peptide over the course of primary CMV infection, but show that early responses may exhibit relatively focused antigen specificity and lower peptide avidity.

**References**
Tuesday, November 7th, 2006

Prevention (Session E)
Prevention of congenital CMV infection by serologic screening of pregnant women: a rational approach

SP Adler
Medical College of Virginia Campus/VCU Richmond, VA USA

Conclusions. Several recent observations make screening of pregnant women for a primary CMV infection attractive. First is a better understanding of the pathogenesis of congenital CMV disease. Severe disease occurs almost exclusively in women with a primary CMV infection during or just prior to pregnancy. In the US about 50% of pregnant women are seronegative and of these from 1% to 8% will acquire CMV during pregnancy. Of these, 50% will transmit CMV to the fetus and about half of infected fetuses will have disease. Second, sensitive and specific methods exist for serologic diagnosis of a primary CMV infection using either single or serial blood samples. Third, preventive measures have been studied and are safe and appear effective. CMV hyperimmune globulin (HIG) was recently evaluated to prevent fetal infection. For women with a primary infection at < 21 weeks gestation or who refused amniocentesis, HIG (100 U/kg) was offered monthly until delivery. 56% of 126 women who did not receive HIG delivered infected infants compared with a 16% infection rate for 37 women who received prophylactic HIG (p< 0.001). Since preventing maternal infection is preferable to HIG administration behavioral intervention methods have also been evaluated. Several studies determined if protective behaviors prevents child to mother transmission of CMV during pregnancy. Seronegative mothers with a child < 36 months of age attending a large group child care center were studied. Women who were not pregnant, pregnant, or attempting to conceive were randomly assigned by child care center to either a control or an intervention group. Mothers in the intervention groups were given instructions for frequent hand washing, wearing gloves for specific childcare tasks, and for avoiding a variety of types of intimate contact with their child. From several studies, we observed that only 1 of 31 pregnant women acquired a CMV infection during pregnancy after being given the intervention compared to 60 of 147 non-pregnant women or women attempting to conceive (p<0.0001). Therefore, intervention prior to pregnancy is ineffective, but pregnant women with a child in daycare should be given the option for serologic testing. Taken together all of these observations support the need for expanded field trials to confirm the importance of universal screening of pregnant women for a primary CMV infection.
ORAL COMMUNICATIONS
E-22. Women’s knowledge of congenital cytomegalovirus

MJ Cannon, DS Ross, M Victor, E Sumartojo

Centers for Disease Control and Prevention, (CDC) Atlanta, GA, USA

Background/Objectives. To better understand how to design prevention messages and strategies, we assessed women’s knowledge of congenital cytomegalovirus (CMV) and what women might be willing to do to prevent congenital infection.

Methods. We submitted four questions to HealthStyles™, an annual postal mail survey sent to a large sample of adults 18 years of age or older (> 4,000/year) in the United States. The four questions asked whether respondents had heard of CMV, where they had heard of it, what they knew about its effects, and what they would be willing to do in order to prevent congenital infection.

Results. Preliminary results show that more women (16%) than men (11%) have heard of CMV, fewer women 18–24 years of age (14%) and older than 65 years of age (12%) have heard of CMV than women 25–64 years of age (16–18%), and that fewer women of Hispanic origin have heard of CMV (11% versus 17–19% for other racial and ethnic groups). Of women who have heard of CMV, most heard about it through a health care provider or clinic (28% versus 2% to 13% for other types of communication). In addition, women who have heard of CMV might not always have accurate information; however, they do appear to know it is serious.

With the knowledge that an infection can hurt their unborn child, most women (98%) would find it easy to wash their hands after changing a child’s diaper and easy to avoid sharing utensils with young children (86%). Most women would find it easy or somewhat easy to avoid kissing a young child on the mouth (69%), although a large minority (31%) would find it somewhat hard or very hard.

Conclusions. Education about prevention of congenital CMV is needed. Women appear to obtain their knowledge from health providers and clinics, which helps to identify the best places to disseminate information about prevention. Handwashing and avoidance of sharing utensils will likely be easy messages to get across. Age differences might affect how the messages are accepted. The next steps in developing a prevention campaign would include using these results to begin developing consumer-based prevention messages and materials, and then testing the materials with focus groups.
Interventions against pre-neonatal infections need to be carefully planned and executed. Over the past fifteen years, European multi-centre studies have tried to compile data from observational studies in order to provide data for rational decision on pre- and neonatal screening programmes for congenital toxoplasmosis. There are many problems in studies of therapeutic interventions against intrauterine infections and the beneficial effect of screening programs for *Toxoplasma gondii* still remains controversial. One problem with previous observational studies was a lack of strict stratification according to gestational age of infection. Because maternal–foetal transmission vary greatly with gestational age a strict stratification of cases and controls are necessary. Also in maternal CMV infection foetal transmission seem more likely to result in clinical symptoms in the beginning compared to later in pregnancy. Recruitment of cases is another important factor as referral centres are likely to receive patients suspected of congenital infection, which may easily result in a referral bias. Diagnostic procedures and protocols for the diagnosis of maternal and foetal infection as well as clinical investigation and follow up of the child have to be adequate and standardised. Confounding by indication arise when a treatment is given for reasons associated with the outcome of interest. If for instance anti-CMV treatment is given for suspected intracerebral changes not caused by CMV. Procedures such as ultrasound scans of the brain and other organs are difficult to validate and to avoid bias it is important that the diagnosis is blind to the investigator. The way forward in intervention trials of new treatment of CMV infection in pregnancy and newborn is multicentre placebo controlled trials, ideally double blinded. From the Eurotoxo project it is evident that collaboration of many centres and experts in clinical virology, obstetrics, foetal diagnosis, pediatrics, radiology, audiology, ophthalmology and epidemiology is required. Furthermore the variation in epidemiology over time and between populations must be taken into account before screening programs are introduced and the effect has to be followed by surveillance programs.
E-44. Congenital CMV infection in HIV infected women on HAART

M Barbi, G Ferraris,* S Binda, S Caroppo, V Primache, A Mammoliti,
AM Bucceri,* L Pugni,* F Mosca*

Dept. Public Health, University of Milan, Italy; *NICU, Cl. L. Mangiagalli, University of Milan, Italy

Background. The introduction of antiretroviral therapy (ART/HAART) has modified both the natural history of HIV disease and the epidemiology of CMV disease in HIV patients by lowering the rate of CMV reactivation. Anti retroviral therapy is recommended in HIV infected women in order to reduce the risk of vertical transmission of HIV. To verify whether ART/HAART can have some preventive effect also on the vertical transmission of CMV we performed a survey of congenital CMV infection in a cohort of HIV infected pregnant women on ART/HAART.

Methods. We examined 303 consecutive children born in the years 2000-2005. The mothers received lamivudine plus zidovudine since week 22 (13-38) of pregnancy; from 2003 a protease inhibitor was added to the treatment; their CMV serological status was assessed at first visit, HIV viral load and CD4/CD8 counts were measured at delivery. All newborns were screened for congenital CMV infection by means of viral isolation and/or CMV PCR on saliva samples collected in the first 3 days of life. Urine and PBL of infected babies were examined in the following days and at clinical follow-up visits.

Results. Vertical transmission of CMV occurred in 9 cases (2.97%), HIV was transmitted in two other babies only (0.6%). Four of the CMV infected children were premature, one of them was symptomatic at birth, one developed a monolateral hearing loss at age 4. None of the term babies was symptomatic at birth nor has developed any sequela so far. Only the mother of the symptomatic girl had a positive IgM test, all the other transmitting mothers were IgG+/IgM- in pregnancy.

Conclusions. 1) Congenital CMV rate is about 10 times higher than in the open Italian population (0.2%), but lower than the one (5.7%) found in a previous Italian study on babies born to HIV infected mothers; 2) The lower rate of transmission might be due to the reduction of CMV reactivation caused by ART/HAART.
Tuesday, November 7th, 2006

Round Table on CMV vaccine
Animal Models for Evaluation of CMV Vaccine Strategies

MR Schleiss

University of Minnesota Medical School, Center of Infectious Diseases and Microbiology
Translational Research, Minneapolis, MN, USA

In consideration of the animal models used to study CMV vaccines, both rodent and primate models have been employed. The guinea-pig model is uniquely useful amongst the rodent models, because of the ability of the guinea pig CMV to cross the placenta and infect the fetus. Observations made during the course of vaccine studies in this model, and other relevant models, will be reviewed. Study of CMV vaccines in these models may help in prioritizing future human clinical trials of vaccines for prevention of congenital CMV infection.
Augmenting immunity to CMV with DNA vaccines

RB Moss

Vical Incorporated, San Diego, CA, USA

An effective vaccine against congenital human cytomegalovirus infection will require effective pre-conceptual immunity in women of child-bearing potential. Such immunity should directly prevent infection or reduce viral replication in order to decrease transmission. The ideal vaccine should elicit both humoral and cell-mediated immune responses. A number of attributes of DNA vaccines make them viable candidates to impact congenital CMV infection. DNA vaccines can target multiple, specific CMV proteins including gB, pp65, and IE1, which may be pertinent in order to elicit a broad immune response. Phase 1 clinical trials with Vical’s DNA vaccines which will be presented and suggest that they are safe and immunogenic. An ongoing Phase 2 clinical trial in hematopoietic stem cell transplant patients with DNA vaccination is currently ongoing. Furthermore, prime-boost or pseudo challenge approaches are being examined with CMV DNA vaccines in clinical trials in order to enhance virus-specific immunity and to substantiate the priming of immunological memory induced by DNA vaccination. Data from prime-boost or pseudo-challenge studies involving DNA vaccination will be reviewed. Future trials are being planned to determine the potential impact of eliciting immune responses by DNA vaccination in order to protect as well as decrease viral load after CMV infection.
Strategies using attenuated poxviruses to confer protective immunity to 
CMV after transplant and potential role as a prophylactic vaccine for congenital infection

DJ Diamond, Z Wang, C La Rosa, T Srivastava, W Zhou, WJ Britt

Laboratory of Vaccine Research, Beckman Research Institute of the City of Hope, Duarte, CA and
Department of Pediatrics, University of Alabama, Birmingham, AL, USA

Human CMV continues to be a major risk factor in patients undergoing allogeneic hematopoietic stem cell (HSCT) and solid organ transplant (SOT), despite advancement of antiviral therapy. CMV tegument protein pp65 and CMV immediate early gene product IE1 are both considered immunodominant targets of cell-mediated immunity (CMI) and potentially capable of controlling CMV infection. Similar to healthy adults, CMV tegument protein pp65 and IE1 are frequently recognized by human CD4 and CD8 T cells in HCT and SOT recipients. To better assess their role in host defense, we have constructed a novel attenuated poxvirus (MVA) transfer vector named pZWIIA and generated a recombinant MVA (rMVA) expressing both full-length pp65 and exon4 of IE1 (pp65-IE1-MVA) at high levels, followed by the genetic removal of the bacterial marker gene used to distinguish recombinant forms during the screening process. Immunogenicity evaluation indicates that pp65-IE1-MVA not only can induce robust primary CMI to both antigens in HLA A2.1, B7 and A11 transgenic mice, but also can stimulate vigorous expansion of memory T lymphocyte responses to pp65 and IE1 in PBMC of CMV-positive donors. The recent discovery that additional early antigens of CMV are also well-recognized by healthy adults prompted us to include the IE2 antigen in the vaccine. Evaluation of in vivo immunogenicity of the vaccine expressing IE2 in both transgenic mice and in vitro antigenicity in human peripheral blood lymphocytes of HSCT and SOT recipients will be presented. A novel overlapping peptide library spanning full-length IE2 has been constructed in our laboratory, and used in conjunction with the vaccine expressing IE2, along with IE1 and pp65. These properties make the MVA-based vaccine ideal for the dual role of priming and boosting CMV-specific T cell immunity as a means to control CMV disease in recipients of HCT or SOT. Substitution or addition of antigens which elicit humoral immunity to neutralizing determinants can expand the utility of this vector system towards prophylaxis of congenital infection. The versatility of MVA and the availability of multiple cloning sites for additional antigens makes it an attractive option for developing immunity to a combination of humoral and T cell response antigens. pZWIIA alone or in combination with other MVA transfer vectors can be used to generate MVA based multiple-antigen vaccine which have application in vaccine development for a wide spectrum of infectious diseases and cancer.
Tuesday, November 7th, 2006

Therapy (Session F)
Antiviral therapy for CMV infections has been effective in preventing and modifying CMV disease in immunocompromised patients. However, evidence for efficacy of antiviral therapy for congenital CMV infection is only modest. Reports of treatment experience with congenital CMV infection must be viewed in relation to what is known about the natural history of congenital CMV disease. The outcome of congenital CMV infection is highly variable and it often takes years to determine the impact of the congenital infection on central nervous system function. Infected infants usually shed virus in multiple body fluids for several years. The rationale for treatment of newborns with congenital CMV infection is based primarily on the fact that hearing loss often appears or worsens after birth, and the best evidence of efficacy of antiviral therapy is from a randomized, placebo controlled study of newborns with severe symptomatic infection in which hearing was the primary endpoint. Whether the outlook for other sequelae such as mental retardation or cerebral palsy can also be improved by antiviral treatment remains to be determined. Recent reports of success with passive immunization during pregnancy to prevent or treat fetal infection suggest that prenatal therapy, given at the time when much of the fetal damage from CMV is occurring merits further study. Improvements in therapy for congenital CMV infection will require agents that can be given at a time that damage can be prevented or arrested and can be continued through the period of life in which ongoing virus replication could lead to further damage. Because of the variable outcome of congenital CMV infection, scientifically rigorous design of studies of new therapies will be essential.
F-03. Ganciclovir therapy of congenital cytomegalovirus infection in symptomatic infants

EM Ruga,1 A Suppiej,2 L Zancan,3 G Guariso,2 E Rizzardi,1 F Farina,3 R D'Elia1

1Division of Pediatric Infectious Diseases, Department of Pediatrics,
2Department of Pediatrics University of Padova, Padova, Italy

Background/Objectives. Ganciclovir has been recently reported to be clinically effective in the treatment of symptomatic congenital cytomegalovirus (CMV) infection. Clinical aspects of the infection in 8 symptomatic congenitally infected children all treated with i.v. ganciclovir are described.

Methods. Eight of 15 symptomatic congenitally infected children seen at our Department were treated with i.v. ganciclovir: medical records were reviewed. Findings at birth included microcephaly (five of eight), intracranial calcifications (five of eight), petechiae (eight), hepatosplenomegaly and hepatitis (eight). First audioligic assessment was performed by auditory brainstem response (ABR) within 2 months from birth (range 7-59 days). All children had at least two ABR. Ganciclovir (12-15 mg/kg/day) was started at a median age of 2 days (range 2-33 days) with a median duration of 42 days (range 22-128 days). The follow-up duration ranged from 9 to 120 months (media 48 months).

Results. Spasticity, developmental delay and/or seizure were observed in all five children with microcephaly and intracranial calcifications. Two of 8 children developed cirrhosis, one of whom received liver transplantation at six year of age because of severe portal hypertension; both showed skin purpura like lesions that persisted for several months after birth. At first audiologic assessment, 1 child had normal hearing and 7 children had sensorineural hearing loss (SNHL). Five of 7 had profound SNHL (2 of 5 unilateral) and 2 severe SNHL (1 of 2 unilateral). Three of 5 children with profound SNHL had sepsis at birth, two of them without neurological findings. A six-month ABR follow-up demonstrated no progression of SNHL in the 2 children with severe loss and absence of hearing impairment in the normal hearing child. No progression from unilateral to bilateral SNHL was observed. The ganciclovir dosage was decreased and then discontinued intermittently in one of 8 patients because of neutropenia.

Conclusions. In congenitally cytomegalovirus infected children ganciclovir therapy may prevent progression of SNHL from severe to profound and from unilateral to bilateral. Moreover our data suggest that ganciclovir do not influence the progression of CMV liver disease.

References

F-43. Maternal administration of valacyclovir in symptomatic intrauterine cytomegalovirus infection

F Jacquemard,1 M Yamamoto,1 J-M Costa,4 S Romand,2 E Jaqz-Aigrain,3 A Dejean,1 F Daffos,2 Y Ville1
1Service de Gynécologie Obstétrique de Poissy-St-Germain, France; 2Service de Médecine fœtale, Institut de Puériculture de Paris, France; 3Service de Pharmacologie, Hôpital R. Debré, Paris, France; 4Service de Biologie Moléculaire Marcel Dassault, American Hospital of Paris, Neuilly, France

Objective. To report early experience with treatment of intrauterine cytomegalovirus infection with maternal oral administration of valacyclovir (VACV).

Study design: Pregnancies with confirmed fetal cytomegalovirus infection were treated with oral VACV (8 g/day). Fetal viral load and drug concentration were monitored in amniotic fluid and in fetal blood.

Results. Twenty pregnancies including 21 fetuses were treated at 27.4±3.2 weeks (range: 22 to 34) for 6.3±3.5 weeks (range: 1 to 12). Ten infants are developing normally at between 1 and 5 years of age. Two infants (both 2 years) have severe unilateral deafness. One neonate presented with microcephaly and severe deafness but was also diagnosed with incontinentia pigmenti. Six out of 7 cases that eventually requested termination of pregnancy had evidence of in utero progression of the disease with worsening cerebral lesions. One fetus died in utero. Therapeutic VACV concentrations were achieved in maternal and fetal blood. The viral load in the fetal blood decreased significantly after 1 to 12 weeks of treatment (U Mann Whitney p=0.006).

Conclusions. Intrauterine treatment of fetal CMV infection may be possible with maternal oral administration of valacyclovir. Our results suggest that in cases where termination of pregnancy is declined, a randomised controlled study to further study this treatment option may be warranted.
POSTERS

Pathogenesis (Session A)
A-07. Congenital cytomegalovirus infection and newborn hearing screening

L Dornier,1 C Czajka,1 A Chaurand,1 A Coquelle,2 C Guillermet,3 N Khayat,4 F Paratte,4 JC Chobaut,1 L Tavernier1

1ENT; 2Virology; 3Paediatrics, CHU-Besançon, France

Background. Diagnoses of hearing loss have been carried out more systematically since the studies on mutations of many genes (connexin mutation and other) (Morton and Nance, NEJM 2006; 354: 2151–64). Different causes of sensorineural hearing loss in children have been better identified. Among the environmental causes, we find congenital infection caused by cytomegalovirus (CMV) (Barbi et al, JCV 2006; 35: 206–9) but the prevalence of this infection in deafness is not really known (Nance et al., JCV 2006;35:221–5). It is nevertheless thought to be the major cause of acquired deafness.

Methods. At the Besançon University Hospital, we carried out a systematic newborn hearing screening of children at risk, in premature births and children in intensive care. The protocol consisted of a AOE test realised as far as possible. A second test was performing in case of failure, one or two days after. And ABR in case of negative response at both, before the child is discharged. 970 children were tested between April 2001 and March 2005.

Results. 10 cases (0.97%) of profound deafness were identified and 21 medium level (sensorineural or conductive) hearing loss. 6 cases (19%) of congenital CMV infection were proven : 5 profound (50%) and 1 bilateral medium (4,8%) sensorineural hearing loss. The CMV infection was diagnosed in one case on amniotic liquid (amniocentesis realised for microcephalia), by maternal serology during the pregnancy or by virus isolation in urines within the first 15 days of life. 4 children were males (2 twins), 2 females. On the 5 profound, 3 were bilateral and 2 unilateral. The reasons of inclusion in this protocol were prematurity in 4 cases, one persistent hypoglycemia and one severe neonatal infection. The 3 profound bilateral hearing loss were fitted with CI (cochlear implant) at the age of 2, 2.5 and 4 (this last one was a progressive hearing loss). In all cases at the MRI: some abnormalities were found on white matter of brain, 4 had neurological troubles.

Conclusions. This kind of sensorineural hearing loss is variable, uni or bilateral, medium or profound, present from birth or evolving. It would be interesting to precise the prevalence of the rate of congenital CMV infection in the population of the deaf children we have studied. For this purpose, we suggest the systematic research using the CMV PCR method with blood samples from Guthrie cards. Different additional procedures can also probably be carried out.
A-09. Post traumatic transmission of cytomegalovirus (CMV) to the foetus and congenital CMV infection

A Coaquette, C Guillermet, F Paratte, N Khayat, G Herbein, L Dornier, D Amsallem, A Menget

Paediatrics, Virology, ENT, CHU Besançon, France

Background. The present case of CMV transmission to the fetus after a maternal traumatism may enlighten the question of placental barrier and its role in CMV fetal contamination.

Method. We investigate the clinical and virological data of woman, victim of a traumatism at 30 weeks of pregnancy without previous abnormality.

Results. A pregnant woman with post traumatic retro placental hematoma and acute foetal distress requires a delivery with cesarean section. The newborn shows anemia, major hypotonia and respiratory distress syndrome. Early bacterial infection, anemia, blood pressure disorders and neurological abnormalities improved. Breast milk diet is allowed. Symptoms of infectious mononucleosis (IM) appear after 6 weeks of life. No viral tests were performed before this term. A massive viruria, up to 100,000 pfu/mL is constantly detected during the following weeks. CMV and EBV viremia are detected by PCR. CMV IgM and IgG are positive but IgG disappear after 2 weeks. EBV (Epstein Barr virus) IgG are positive without IgM. The maternal serology detects CMV and EBV IgG without IgM.

Discussion. The CMV viruria in this case is a generalized herpes-simplex-like cytopathic effect seen 24 hours post inoculation on MRC5 cells, usually described in symptomatic CMV congenital infection. But clinical controls performed during pregnancy were normal. According to clinical records, CMV congenital infection is not probable. Viral uptake from breast milk or blood transfusion is neither probable because mixed infection (CMV-EBV) is not described after breast feeding and transfused blood was filtered. The most probable route of viral transmission remains the traumatic placenta. Histopathologic placenta examination shows vascular infarcts and bleedings, suggesting the passage of large amount of maternal blood in fetus. This case mimicks an IM after non filtered blood transfusion.

Conclusions. This report recalls that placental barrier can be deficient. We use to think that late virus transmission to the fetus represent a mild contamination balanced by the presence of specific antibodies, eventually acting as a natural vaccination. Nevertheless, according to the potential risk of delayed hearing loss in infected children, further studies should try to insure the relative safety of such contaminations. In order to investigate this way, we propose an acceptable procedure to detect CMV immunisation and/or infection in children in their early life.
Background. Distribution of CMV strains can vary according to the geographic region. Investigation if virological markers are associated with transplacental infection can help to understand the pathogenesis of this infection.

Objectives. To verify if the CMV gB genotype is a correlate of intrauterine viral transmission, evaluating the occurrence of coinfection with multiple CMV strains in mothers and their congenitally infected neonates.

Methods. A total of 50 congenitally CMV infected neonates diagnosed among 4439 screened infants (incidence=1.1%) and their 48 mothers were enrolled in the study. Urine and saliva samples were obtained from all infants and dried spot blood samples were obtained from 45 of them. Congenital CMV infection was confirmed by CMV-DNA detection in urine and saliva samples and identification of viruria within 2 weeks of life. Maternal breast milk, urine and saliva specimens were obtained by 3 months after delivery (median=3 days). Genotype analysis was done by restriction fragment length polymorphism of PCR products amplified by primers for the cleavage site of the UL55 gene which encodes glycoprotein B (gB).

Results. Only 2/50 infants (4%) were symptomatic. Genotyping was performed in CMV DNA detected in 50 specimens of urine, 50 of saliva, and 40 of dried blood spots obtained from infants. Overall, maternal CMV genotypes were determined in 46/48 (95.8%) mothers. According to maternal specimens, genotyping was performed in CMV-DNA obtained in 43 breast milk samples, 13 urine samples, and 2 saliva samples. No gB4 was found in mothers or neonates. No predominance of any CMV gB genotype was observed in infants (gB1:19(38%), gB2:18(36%), gB3:13(26%); p=0.39) or in mothers (gB1:15(33%), gB2:18(39%), gB3:13(28%); p=0.54). A single genotype was identified in urine, saliva and blood from each of the infected neonates. The same maternal breast milk genotype was found in urine, saliva, and blood of the respective offspring. Three mothers were infected with more than one genotype genotypes (gB1/gB3; gB3/gB2; gB1/mixture of gB). However, only one of these was found in their infants (gB3, gB3, gB1, respectively).

Conclusions. CMV genotypes distribution in Brazilian mothers and infants likely reflects the overall frequency of viral strains circulating in the general population. No favorite strain is being transmitted from mother to fetus. Based on CMV gB genotypes, maternal coinfection with different strains is uncommon, and a single genotype is transmitted to the infant.
A-46. Expression of human cytomegalovirus immediate early genes disrupts normal development in a transgenic drosophila model

R Steinberg, Y Shemer, S Silberberg

Department of Virology and Developmental Genetics, Ben-Gurion University, Beer-Sheva, Israel

Background. Intrauterine infection with human cytomegalovirus (HCMV) is the leading viral cause of birth defects involving the central nervous system. Due to the highly species specific nature of HCMV, its course of natural infection cannot be studied in animal models. The virus immediate-early (IE) genes are the earliest genes expressed upon infection. Their products regulate viral gene activation and host responses to infection.

Aim. Here we introduce a novel transgenic Drosophila melanogaster model system for studying the effects of the IE genes on normal embryonic development.

Methods. For this purpose we generated transgenic flies carrying the HCMV IE genes under the control of a hsp70 promoter for ubiquitous ectopic expression, or under a tissue specific inducible promoter (using the UAS/GAL4 inducible expression system) that enables us to study the effects of IE gene expression on specific tissues, such as the nervous system. We monitor phenotypes upon ectopic IE expression and characterize molecular event involved.

Results and Conclusions. Our results suggest that ectopic expression of the IE genes during embryogenesis is associated with increased embryonic lethality. Unhatched embryos display morphological abnormalities associated with defects in adhesion of epithelial cells. In addition, ectopic expression of the IE genes in the developing eye disrupts eye specification and patterning. Owing to the high degree of evolutionary conservation between mammals and invertebrates, it is expected that the fly studies will be also relevant and applicable to humans. Furthermore, we anticipate that this system will be useful in studying many other aspects of viral-host interactions.
POSTERS

Diagnosis in the mother, fetus and newborn (Session B)
Laboratories play a very important role in the diagnosis of congenital and perinatal CMV infection, considering that other viral infections in newborn infants have similar clinical characteristics.

From a total of 148 patients, 296 samples of dry blood from the newborn heels were analyzed by PCR, whereas urine samples were studied by PCR and viral isolation.

The samples from 19 patients, taken at birth, were studied between one and eight months later. The objective of this work is to compare the PCR results of eluted dry blood with the viral isolation in tissue culture and the urine PCR, stating the advantages of each method.

From the 148 patients, 50 presented CMV infection, 3 had other viral infections and 95 tested negative.

From the 50 positive CMV patients, 35 had congenital infection. Thirty-one of them were diagnosed in the first 15 days after birth, whereas four of the ones who showed symptoms after birth were studied by PCR in urine and retrospectively in dry blood. The dry blood and urine PCR samples showed 100% sensitivity, whereas viral isolation sensitivity reached 68.57%.

The urine specimens from the remaining 15 patients that were taken between 1 and 8 months after birth, and which had CMV infection, were analyzed by tissue culture isolation and by PCR showing a sensitivity of 60% and 100% respectively. The retrospective analysis of this dry blood group yielded negative results so the infection was considered perinatal.

In conclusion, the dry blood PCR of the newborn infants makes it possible to confirm the presence of a congenital CMV infection even if the sample taken at birth is analyzed later. This is very important in those cases of late symptomatology. Another important advantage is that the samples may be sent to distant places without special conditions.

Key Words. Cytomegalovirus – congenital infection – perinatal infection.
B-14. CMV congenital infection: results of a prospective study

S Lopo, P Palminha, M Pité, T Caçador, E Vinagre, M Pereira, T Paixão, RM Brito, J Garrote, H Carreiro, C Machado

*NIH, Lisbon, Portugal; †Fernando Fonseca Hospital, Lisbon, Portugal

**Background.** Cytomegalovirus (CMV) is considered the most frequent cause of congenital infections, occurring in approximately 0.4–2.0% of all live births, being responsible for neonatal mortality and morbidity. Several newborns with congenital CMV infection lack clinical symptoms at birth but may show late sequelae, so that infection is easily overlooked. Therefore, these infections are neither diagnosed nor treated. The Centre of Virology of the NIH reports the results of a prospective study on the diagnosis of congenital CMV infection in a group of consecutive neonates from a Hospital located in Lisbon.

**Objective.** To determine the proportion of CMV congenital infection in a group of consecutive neonates, during three months. To determine the sequels of CMV congenital infections, after three years of clinical and laboratory attendance.

**Methods.** We determine the proportion of CMV congenital infection in a group of 757 consecutive neonates between May-August 2002; the laboratory diagnosis was established by detection of CMV in cell culture (shell-vials) from urine, during the first 2 days of life. PCR was performed in 653 cases from which cord blood was collected and in the urine of those whose shell-vials was positive. Serological tests in sera, viral detection from urine and paediatric follow-ups were performed periodically in cases of intrauterine infected neonates. The mothers of the congenitally infected neonates were screened for IgG and IgM antibodies after being informed about the laboratory results of their infants.

**Results.** Congenital CMV infection was diagnosed in 5 of 757 cases (0.7%) all asymptomatic at birth. In all cord blood samples CMV viral genome was not detected. Two newborns (2/5–40%) showed signs/symptoms of congenital CMV disease during the first month of life (pneumonia, vasculitis, bone marrow failure, hepatitis). The other 3 cases were asymptomatic during the first months but showed late manifestations (short stature, partial hearing loss, limphocytosis).

**Conclusions.** There was no prior knowledge about the incidence of CMV congenital infected children in Portugal but in this prospective study the proportion of intrauterine transmission is in agreement with literature. The five infected newborns were followed during the first 36 months of age and in all were detected minor manifestations. The value of screening young women for CMV is still controversial; however it may help in the prevention of congenital infections.
B-16. A study of congenital and postnatal cytomegalovirus (CMV) infection in the neonatal unit at the Royal London Hospital, United Kingdom

TN Brown,1 A Sinha,1 F Mattes,2 I Ushiro-Lumb,2 ST Kempley1
1Dept of Neonatal Medicine; 2Dept of Virology, Barts and The London NHS Trust, United Kingdom

Background. Congenital CMV infection is usually asymptomatic at birth but 5–15% of these infants are reported to have abnormal development and/or sensorineural hearing loss later in life. In 1995, a screening programme was introduced to detect congenital CMV infection in babies admitted to the neonatal unit at the Royal London Hospital. There is limited information on effects of early postnatal CMV infection in extremely preterm infants.

Aim. To determine the incidence, clinical characteristics, and outcome of infants with congenital and postnatally acquired CMV in babies admitted to our neonatal unit.

Methods. All positive CMV results (Detection of Early Antigen Fluorescent Foci - DEAFF test in urine) for babies admitted to the NICU at the RLH between January 1999 and June 2005 were identified from the virology database. These included results from screening (tested within 21 days of life to exclude congenital CMV) as well as babies tested beyond 3 weeks from birth due to clinical symptoms (postnatal CMV). The demographics, clinical features and outcome data for these babies was collected and compared.

Results. Out of 1803 babies admitted to NICU, 12 (6.7/1000 admission) were diagnosed with congenital CMV, and 8 with postnatally acquired symptomatic CMV. The results are summarised in the table below. Babies with postnatally acquired CMV were of lower gestation and birthweight.

Conclusions. Both congenital and acquired CMV in this cohort appear to have significant adverse outcomes. A more detailed analysis including non-CMV infected controls is being undertaken to better assess the impact of CMV infection in this group of vulnerable babies, particularly when infection occurs at early stages of postnatal life.

<table>
<thead>
<tr>
<th></th>
<th>Congenital (n=12)</th>
<th>Acquired (n=8)</th>
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<tbody>
<tr>
<td>Gestation (weeks)</td>
<td>34 (24-40)</td>
<td>25.5 (23-29)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.4 (0.49-2.5)</td>
<td>0.62 (0.46-0.92)</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IUGR 9 (75%)</td>
<td>Microcephaly 7 (58%)</td>
<td>Microcephaly 5/7 (71%)</td>
</tr>
<tr>
<td>Hepatomegaly 3 (25%)</td>
<td>Hepatomegaly 3 (38%)</td>
<td>Splenomegaly 2 (25%)</td>
</tr>
<tr>
<td>Splenomegaly 2 (17%)</td>
<td>Thrombocytopenia 6 (50%)</td>
<td>Thrombocytopenia 7 (88%)</td>
</tr>
<tr>
<td>Outcome (follow-up &gt; 6 months)</td>
<td>Died 1/12 (Cause - klebsiella sepsis/CLD)</td>
<td>Died 2/8 (Cause – 1. CLD, Cong abnormality, 2. CLD, Pul Hypertension Abnormal development 3/6 (50%)</td>
</tr>
<tr>
<td></td>
<td>Abnormal development 5/9 (56%)</td>
<td>Abnormal development 3/6 (50%)</td>
</tr>
<tr>
<td></td>
<td>Sensorineural deafness 0/9</td>
<td>Sensorineural deafness 0/6</td>
</tr>
<tr>
<td></td>
<td>Normal outcome 3/9 (33%)</td>
<td>Normal outcome 1/6 (17%)</td>
</tr>
</tbody>
</table>
B-17. Audit of a 10 year period (1995 to 2005) congenital CMV screening in the special care baby unit, Royal London hospital

Z Syed,¹ F Mattes,¹ A Sinha,² I Ushiro-Lumb¹
¹Departments of Virology, ²Neonatal Medicine, Barts and The London NHS Trust, London, United Kingdom

Background. The Royal London Hospital is a teaching hospital which serves a deprived inner London health district, where the incidence of neurosensory hearing loss in children is higher than the UK average. In 1995 a congenital CMV screening programme was introduced in the SCBU. All babies admitted to the neonatal unit should be screened for the presence of CMV in urine within 21 days of life.

Aims of the audit
- To verify compliance with the screening programme.
- To obtain cumulative data on the incidence of congenital CMV infection in the babies tested over this 10 year period.
- To provide information for a follow up audit to look at outcomes of CMV infected babies.

Methods. Urine samples were tested by Detection of Early Antigen Fluorescent Foci (DEAFF) as per standard protocol. An admission list was obtained from the SCBU's database. Babies older than 21 days at the time of admission were excluded. A further list of all the babies tested by CMV DEAFF in urine was obtained from the Virology Laboratory System. The data was merged and analysed.

Results. A total of 2847 babies were admitted to the SCBU during the period studied. 1973 (69%) of those were tested for CMV during their hospital admission, with the absolute majority (95–99%) tested within 21 days from birth. Seventeen babies were identified as congenitally infected (0.88% of the total tested), and an extra 5 were identified as CMV infected after 21 days of life.

Discussion and conclusions. The results indicate that a significant proportion of babies admitted to the neonatal unit are not being screened for CMV (20–50%, depending on the year). There has also been no significant increase in the proportion of babies tested for CMV over the years. The majority of babies are tested within 21 days (95 to 100%). A very small proportion are tested at later stages (0–5%), particularly when CMV infection is suspected. A method is required to ensure that all babies admitted to the neonatal unit are tested for CMV. This might involve education of medical staff on the importance of recognition of CMV infection and early testing. It may also be beneficial to re-screen high-risk babies (extreme premature or low birth weight) at a later stage during admission to identify either babies missed during initial screening or cases of postnatally acquired infection.
B-18. Congenital CMV infection and sensorineural hearing loss: need of identification of asymptomatic newborns

MR Contiero, MP Bellagamba, R Chierici

Neonatal Intensive Care Unit and Neonatology S. Anna Hospital of Ferrara, Italy

Background. Cytomegalovirus is a common cause of congenital sensorineural hearing loss (SNHL). The prevalence of congenital CMV infection is about 0.2–2.2% of newborns. Of congenitally infected CMV infants, 90% are asymptomatic at birth; of these, 10–15% will go on to develop SNHL that may be delayed in onset, unilateral or bilateral, stable or progressive and variable in severity. The infection is clinically apparent in 10% of infants; of these, 30–65% will develop SNHL that is usually early in onset and of greater severity than in asymptomatic children. Late-onset SNHL continues to occur until 72 months of age both in symptomatic and asymptomatic infants.

Objectives. At S. Anna Hospital of Ferrara, from January 2001 to December 2004, we identified 4 infants with congenital CMV infection in a cohort of 5016 newborns. Of the babies infected, one was symptomatic and 3 asymptomatic at birth. These babies were identified by detection of CMV by PCR in urine because the mothers had had seroconversion to CMV during pregnancy. The symptomatic baby was recovered at the Neonatal Intensive Care Unit. He was preterm (33’ weeks) and the clinical manifestations were: petechiae, thrombocytopenia, anemia, hepatosplenomegaly, jaundice, hydrocephalus, sensorineural hearing loss. The asymptomatic infants were submitted to neonatal hearing screening at nursery and, after discharge, to a follow-up in order to detect the occurrence of hearing loss. One presented early-onset bilateral hearing loss, an other one developed monolateral late-onset hearing loss (at 24 months); the last one, actually, has a normal hearing evaluation and will continue the follow-up.

Conclusions. In our population, the prevalence of congenital CMV infection seems to be lower than that described in other studies. Probably, this aspect is due to absence of maternal CMV serology. Because most infants with congenital CMV infection are asymptomatic, the identification of infected babies needs a combined approach of knowledge of maternal serology and neonatal hearing screening. Moreover it is necessary for the asymptomatic children to receive further hearing evaluations to detect late-onset hearing loss.

References
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B-19. Retrospective diagnosis of congenital cytomegalovirus infection using preserved umbilical cords

M Moriuchi, M Tagawa, H Tanaka, H Moriuchi

Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences; Nagasaki-Prefectural School for the Deaf, Japan

Background. CMV infection is the most common congenital infection in developed countries. It has been reported that not a few patients with congenital CMV infection who were asymptomatic at birth later develop a variety of neurological disorders, but that etiological diagnosis has not been made in most of the cases because of the difficulty in making the diagnosis beyond neonatal period. In Japan, umbilical cord is kept clean and dry as a symbol of mother-child bond, and can be provided as useful materials for retrospective investigation.

Methods. Dried umbilical cords were provided from 35 patients with sensorineural hearing loss (SNHL), one patient with refractory epilepsy and one patient with autistic disorder (AD) and psychomotor retardation. In this study, 19 DNA samples that yielded 103 copies or more of tubulin gene DNA per µl were used for further investigation. Those samples were from 17 patients (9 boys and 8 girls, aged 1 to 18 years) with SNHL, a boy with epilepsy and a boy with AD. CMV DNA was amplified with the iCycler iQ Real-Time Detection Systems (Bio-Rad).

Results. CMV-DNA was detected from 4 patients: a 16-year-old girl with SNHL, a 4-year-old boy with SNHL, a 4-year-old boy with epilepsy and a 4-year-old boy with AD. The first patient was never suspected of congenital CMV infection because of a lack of other clinical manifestation. Perinatal history of the second patient was significant only for intrauterine growth retardation (IUGR). Congenital CMV infection was suspected for the third patient at birth based on microcephaly and IUGR; however, CMV-specific IgM was negative and further evaluation such as viral isolation was not made. The fourth patient had been treated as typical AD until he developed afebrile convulsion.

Conclusions. Recently, Guthrie cards have been shown to be useful as materials for retrospective diagnosis of congenital CMV infections. Unfortunately, however, Guthrie cards are stored for only a year or two in many countries including Japan; therefore, retrospective diagnosis of congenital infections is extremely difficult beyond infantile period. Umbilical cord is detached from newborn babies within a week or two after birth; therefore, presence of CMV DNA in the umbilical cord samples should indicate congenital infections. This unique material may provide opportunities for clinicoepidemiological studies of congenital infections.
B-20. Retrospective diagnosis of congenital cytomegalovirus infection in patients with sensorineural hearing loss using the perilymphatic fluid

M Tagawa, M Moriuchi, H Takahashi, H Moriuchi

Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences; Department of Otolaryngology, Nagasaki University Hospital, Japan

Background. Congenital CMV infection is the leading non-genetic cause of sensorineural hearing loss (SNHL). However, it is extremely difficult to make diagnosis of congenital CMV infection when SNHL is ascertained later in life. In addition, diagnosis of congenital CMV infection per se cannot confirm its etiological role in SNHL, because most of congenitally infected individuals remain asymptomatic for lifelong period. Detection of CMV-DNA in the perilymphatic fluid collected at the time of cochlear implantation from patients with SNHL will strengthen its pathological importance.

Methods. A total of 24 patients underwent cochlear implantation at Nagasaki University Hospital, and 5-10 µL of the perilymphatic fluid was collected. DNA was extracted from 2-µL perilymphatic fluid and eluted in 150 µl elusion buffer. In this study, 10 µL of the DNA solution (corresponding to 0.13-µL perilymphatic fluid) was subjected for real-time PCR of tubulin gene DNA to confirm quality and quantity of DNA. Samples from 20 patients (7 males and 13 female), aged 2 to 78 years, contained amplifiable DNA, and were further evaluated for the presence of CMV DNA with the iCycler iQ Real-Time Detection Systems (Bio-Rad). The lower limit of sensitivity of this assay was approximately 20 copies per µl perilymphatic fluid.

Results. CMV-DNA was detected at 1.5×10^2 copies per µL perilymphatic fluid in one of the 20 patients. This 15-year-old boy was asymptomatic during neonatal period and ascertained to have hearing loss during late infantile period. He was not suspected of congenital CMV infection because of a lack of other clinical manifestation.

Conclusions. CMV DNA has been detected in 5 patients (aged 1-4 years) with congenital CMV infection in the previous studies (Sugiuera et al. J Med Virol 2203;69:72-5; Bauer et al., Laryngoscope 2005;115:223-5). Our study suggested that CMV may persist for as lon as 15 years after birth. Although it may not be sensitive enough due to quite small amount of DNA obtained, detection of CMV-DNA from the perilymphatic fluid using real-time PCR is helpful for retrospective diagnosis of SNHL secondary to congenital CMV infection.
B-24. Optimisation of retrospective diagnosis of cytomegalovirus congenital infection from dried blood spots

C Vauloup-Fellous, O Picone, P Dubreuil, L Grangeot-Keros

1 Service de Microbiologie; 2 Service d' Obstétrique Hôpital Antoine Béclère, Clamart, France

Background/Objectives. Out of the 90% of cytomegalovirus (CMV) congenitally infected children that are asymptomatic at birth, 5 to 15% will later develop complications (neurodevelopmental defects and/or deafness). Unfortunately, after the first 2 weeks of life, usual diagnostic techniques for CMV detection (viral culture and serology) are useless to differentiate congenital infection from post-natal acquired infection; whereas detection of viral DNA from dried blood spots (DBS; Guthrie cards), systematically collected from all newborns in the first days of life, has been described for late diagnosis of CMV congenital infection. The aim of our study was to choose and optimise a viral DNA extraction method from DBS, to assess the sensitivity and specificity of the method with DBS collected from newborns with or without a diagnosis of congenital infection based on viral isolation from urine at birth, to study if CMV DNA detection is reliable when DBS are stored for 1 year at room temperature or 2 months at 37°C.

Methods. 8 reference cards (blood collected from CMV seronegative newborns (IgG/IgM negative) were infected with serial dilutions of virus and spotted on Guthrie cards), and 16 Guthrie cards (8 from congenitally infected children and 8 from non infected children) were tested. 3 extraction methods were evaluated, products of PCR were analysed by agarose gel electrophoresis and quantification of CMV from DBS was also performed.

Results. Analysis of the results obtained from reference cards showed higher sensitivity of phenol/chloroform extraction following treatment with proteinase K, compared to heat extraction in cell culture medium or extraction with a commercial kit (Qiagen). CMV DNA was detected in the 8 Guthrie cards collected from congenitally infected children, and not detected in the 8 Guthrie cards from non-infected children. We did not observe quantitative loss of viral DNA after 1 year storage at room temperature or 2 month storage at 37°C.

Conclusions. On condition that high sensitive extraction and amplification methods are performed, CMV DNA detection from DBS could become a very useful tool for retrospective diagnosis of congenital CMV infection, when sequelae are diagnosed in the first years of life. We are pursuing this study with a larger number of DBS from congenitally infected children. Further, we will evaluate the viral load in DBS as a prognosis marker of sequelae in symptomatic and asymptomatic infected newborns.
B-28. Maternal IgM negative antibodies coexisting with fetal ultrasound features of infection do not rule out a congenital CMV infection

A Goncé, O Coll, A Borrell, S Hernández, MA Marcos, J Bosch, E Gratacos

Unitat d’Infeccions Fetals, Institut Clínic de Ginecologia, Obstetricia i Neonatologia, Hospital Clínic, Barcelona, Spain

Background. When fetal ultrasound features of infection are observed, primary CMV is the most suspected agent. Maternal serological diagnosis of primary infection is based on the detection of specific IgM antibodies and subsequently confirmed by CMV detection in amniotic fluid (AF) by DNA amplification (PCR).

Objective and Methods. We report two pregnancies with a severe congenital CMV infection in which maternal IgG was positive at the time of ultrasound findings but IgM was negative. CMV infection was therefore not suspected.

Case reports. 1) A 36 year-old patient, showed fetal ultrasound abnormal findings at 17 weeks’ gestation, at the time of genetical amniocentesis. An hyperechogenic bowel and oligohydramnios led us to screen for congenital infections, with a negative toxoplasmosis and herpes simplex virus (HSV) serology and CMV IgG positive with negative IgM. The patient was assessed for follow-up. At 21 weeks, mild ventriculomegaly with the suspicion of corpus callosum agenesis and ascites were also observed. A second amniocentesis was performed to rule out CMV infection which was confirmed by PCR. The patient requested for termination. Pathological examination confirmed CMV infection. 2) A 21 year-old patient, showed fetal ascites at 20 weeks’ scan with an increased middle cerebral artery peak systolic velocity (>1.5 MoM). Red blood cell alloimmunization was excluded by irregular antibodies test, and the mother was screened for fetal infections. IgG and IgM were negative for Parvovirus B19 and Toxoplasma and IgG was positive for CMV and HSV with negative IgM. A cordocentesis was performed to investigate fetal anemia. Hematocrit was 28% but severe bradycardia leading to fetal demise occured before transfusion was attempted. Pathological examination demonstrated CMV inclusion cells in the lungs, kidneys and liver.

Conclusions. In the presence of ultrasound anomalies suggestive of fetal infection, positive IgG with negative IgM antibodies, cannot rule out a severe CMV infection, presumably acquired at least two months before the appearance of the abnormal sonographic findings. PCR analysis in AF for CMV detection, should be offered in such cases.
B-29. Diagnostic relevance of sensitive assays for CMV IgM in pregnancy

M Gentile, P Pagnotti, P De Marco, A Pierangeli, S Tzantzoglou, C Galli, G Antonelli

Virology and Infectious Diseases, University of Rome La Sapienza; *Medical Marketing, Abbott Diagnostics, Roma, Italy

Background and objectives. CMV infection in pregnant women and in immunocompetent individuals is mostly asymptomatic or accompanied by non specific symptoms. While the presence of CMV-specific immunoglobulin M (IgM) is associated with a recent or active CMV infection, IgM may be positive also in patients with secondary infections or reactivations. Furthermore, commercial methods for CMV IgM show different specificity and sensitivity and a high degree of discordance. We aimed to compare the relative performance of different commercial assays for CMV-specific IgM and evaluate the clinical relevance of IgM positivity in pregnant women.

Methods. Serum samples collected at Policlinico Umberto I Hospital of Rome from January to June 2006 were routinely tested at the Virology department for CMV-IgG and CMV-IgM with AxSYM assays (Abbott Diagnostics). Positive CMV-IgM sera were subsequently tested with CMV IgM and IgG EIA (Radim); on selected patients the avidity of CMV IgG was assessed by EIA (Radim) and virus isolation was performed using a shell vials method on MRC-5 cell culture from urine samples.

Results. Nine hundred-twenty sera were tested for CMV-IgM by AxSYM in the six-months period of the study. Of them, 492 (53.4%) were obtained from pregnant women and among those 95 (19.3%) specimens resulted positive and 54 (11%) greyzone (GZ) by AxSYM. Only 26/149 of the AxSYM reactive specimens (17.4%) were IgM positive also by the Radim assay, while testing by a third CMV IgM method (DiaSorin EIA) is still pending. Thirty-nine subjects with a positive IgM result by AxSYM were further analyzed for IgG avidity and viral isolation; the results are in Table.

Conclusions. Our data confirm the wide variability of the IgM response to CMV as measured by different immunoassays. While a positive result by a sensitive method, such as the AxSYM assay, has a low positive predictive value for a recent infection and requires additional testing for IgG avidity in order to exclude a primary infection, potentially harmful for the newborn, an insensitive test may miss both recent active infections and reactivations with viral shedding, that are common during pregnancy.

<table>
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<tr>
<th>Group</th>
<th>IgG avidity low</th>
<th>IgG avidity high</th>
<th>Shell vial pos</th>
<th>Shell vial neg</th>
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</thead>
<tbody>
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<td>AxSYM and Radim IgM pos</td>
<td>8</td>
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</tr>
<tr>
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</table>
B–30. Performance data of the newly developed completely automatized CMV IgG, IgM and avidity assays on the ARCHITECT platform

J Herzogenrath, HB Braun, R Eichler, H Christ, I Curdt

Abbott Laboratories, Wiesbaden, Germany, Abbott Park, IL, USA

Introduction. A panel of congenital assays for CMV consisting of an IgG, IgM and IgG avidity assay are under development for the ARCHITECT instrument. The main intended use of the panel is the early and reliable identification of primary CMV infections during pregnancy. Sensitivity and specificity data of the single assays will be shown.

Material and Methods. The ARCHITECT CMV IgG and CMV IgG Avidity assay utilize CMV lysate (strain AD169) coated on μ-particles and a conjugate containing an acridinylated IgG Mab directed against human IgG. In addition to particles and conjugate IgG Avidity contains 2 different pretreatment solutions containing aqueous antigen in buffer or buffer only. The avidity assay is based on the AviComp principle a new method for avidity testing. The CMV IgM assay contains virus lysate (strain AD169) and a recombinantly expressed fusion protein with immunodominant epitopes of CMV coated to μ-particles. An acridinylated Mab against human IgM is utilized for detection.

Specificity. The resolved relative specificity and sensitivity of CMV IgG has been determined by testing a population consisting of 500 blood donors, 250 pregnant women, 250 hospitalized patients and 10 transplant recipients (N=1010). For CMV IgM, a population of samples from 300 blood donors, 400 hospitalized patients and 400 pregnant women has been tested. The specificity of the CMV IgG Avidity assay has been determined on 200 CMV immune samples (100 from blood donors, 100 from pregnant women).

Seroconversion Sensitivity. Three commercially available seroconversion panels have been tested on all three CMV assays.

ROC Analysis and cutoff determination. The position of the cutoff has been determined for CMV IgG and IgM by performing a ROC analysis against the resolved result on all tested samples, for CMV IgG Avidity a ROC analysis has been made against the clinical information of tested samples.

Results. All ARCHITECT CMV assays revealed equivalent or better sensitivity and specificity than their reference assays, that have been chosen as reference due to their widely accepted performance.

Conclusions. The performance of all 3 ARCHITECT CMV assays is better or at least equivalent compared to the tested reference assays. The combination of the 3 assays allows reliable routine screening for primary infections. Primary CMV infection are recognized for a slightly longer period of time after seroconversion, resulting in a significantly decreased risk to miss primary infections.
B-31. Development of a completely automated CMV avidity assay for the ARCHITECT instrument using a novel technology with direct detection of low avidity antibodies (AVICOMP)

I Curdt, J Herzogenrath, S Bernhardt, GT Maine, H Christ

Abbott Laboratories, Wiesbaden, Germany, Abbott Park, IL, USA

Introduction. A panel of new CMV assays for the ARCHITECT instrument has been developed including a CMV avidity assay based on the AVicom technology. Intended use of the CMV panel is the identification of primary CMV infections during pregnancy. While conventional avidity assays remove low avidity antibodies by a chaotropic wash, the new AVicom technology removes high avidity antibodies by neutralising with liquid antigen and directly detects low avidity antibodies. Performance data of the newly developed technology will be shown in comparison with clinical data and conventional avidity assays. Furthermore the combined performance of the new ARCHITECT CMV IgG, IgM and Avidity assay will be shown.

Materials and methods. The ARCHITECT CMV Avidity assay contains paramagnetic μ-particles coated with CMV virus lysate (strain AD169). An acridinylated anti human IgG Mab is used for the detection of specific IgG from the patient sample. The assay contains 2 different pretreatment solutions. One consists of aqueous CMV lysate in buffer while the other one contains buffer only.

Avidity testing. Based on the CMV IgG concentration of the sample, the ARCHITECT instrument performs automated sample dilution to adjust the specific IgG content of the sample to the optimal testing concentration. The diluted sample is tested once with each pretreatment solution. The avidity result is calculated from the 2 results. It is expected that seroconversion bleeds are low avidity for at least 4 month after the last CMV IgG negative bleed.

Specificity. The specificity has been determined on 200 samples from CMV immune blood donors.

Seroconversion Sensitivity. 3 commercially available seroconversion panels were tested and compared to Radim and Vidas CMV Avidity

Results. The clinical specificity of the ARCHITECT CMV Avidity assay is at least > 99.2%. The seroconversion sensitivity was 97.2%. The high analytical sensitivity of the new Avidity assay allows earlier and slightly longer determination of low avidity than other on market CMV avidity assays. In combination with ARCHITECT CMV IgG and CMV IgM a specificity of >99.8% with the same sensitivity as CMV Avidity alone has been achieved.

Conclusions. The AVicom avidity format shows higher agreement with the clinical data than with the results of conventional avidity formats. The complete automatization of ARCHITECT CMV IgG, IgM and CMV avidity allows reliable and fast identification of primary CMV infections.
B-32. Prospective evaluation of human cytomegalovirus (HCMV) detection in blood for diagnosis of primary and congenital HCMV infection

MG Revello, M Furione, M Zavattoni, G Gerna
Servizio di Virologia IRCCS Policlinico San Matteo, Pavia, Italy

Objectives. To verify prospectively diagnostic values of HCMV DNA and IEmRNA detection in blood of immunocompetent subjects with suspected primary and fetuses/newborns with congenital HCMV infection.

Methods. From Jan 2004 to Dec 2005, 606 sequential blood samples from: i) 276 immunocompetent individuals (271 pregnant women) with suspected primary HCMV infection; ii) 20 fetuses (11 infected, 9 uninfected); iii) 25 uninfected newborns; iv) 23 congenitally infected infants examined either at birth (n=13) or at later times (n=10), were prospectively tested for HCMV detection in blood by using in parallel house developed nested and quantitative PCR assays and a commercial IEmRNA NASBA kit (BioMerieux). In addition, 54 amniotic fluid (AF) samples from 52 pregnant women with proven primary HCMV infection were tested. Serologic diagnosis of primary HCMV infection was based on seroconversion, kinetics of IgM response, and IgG avidity. HCMV congenital infection was diagnosed in utero by virus isolation from/detection in AF, and at birth by virus isolation from urine samples.

Results. Altogether, PCR and NASBA gave concordant results in 474/660 (71.81%) negative samples and in 99/660 (15.0%) positive samples (overall concordance 86.8%). Discrepant results included 67/660 (10.15%) PCR+/NASBA- samples and 20/660 (3.03%) PCR-/NASBA+ samples. In detail: Group i) primary HCMV infection was diagnosed in 147/276 (53.26%) individuals. HCMV was detected in blood of 44/147 (29.93%) subjects by both assays, in 30/147 (20.4%) by PCR only, in 10/147 (6.8%) by NASBA only, whereas 63/147 (42.85%) subjects were negative by both assays. Two/129 (1.5%) pregnant women with non-primary HCMV infection were PCR or NASBA–positive. Group ii) 11/11 (100%) HCMV-infected fetuses had virus in blood, whereas all 9 uninfected fetuses were negative by both assays. Group iii) All 25 uninfected newborns were negative by both assays. Group iv) 13/13 (100%) and 10/13 (76.9%) HCMV-infected newborns had virus in blood at birth by PCR and NASBA, respectively. Finally, 22/54 (40.74%) AF samples were HCMV-positive by both assays.

Conclusions. i) This study confirms that nested PCR is more sensitive than IEmRNA NASBA in detecting HCMV in blood; ii) HCMV is rarely detected in blood of immunocompetent individuals with non–primary HCMV infection; iv) both assays can be reliably used for prenatal diagnosis of congenital HCMV infection.
B-34. Diagnosis of congenital cytomegalovirus infection in dried blood spots: a 5 year experience in Portugal

S Almeida, P Paixão, P Gouveia, C Vilarinho

1Centro Hospitalar Covilhã, Covilhã, Portugal; 2Faculdade de Ciências Médicas, Lisboa, Portugal; 3Instituto de Genética Médica Jacinto Magalhães, Porto, Portugal

Human cytomegalovirus (CMV) is the most frequent cause of congenital infection. 0.5 to 2% of all live births are infected by this virus. The standard diagnostic test is a viral culture of urine or saliva obtained from a newborn within the first three weeks of life. However, since neurological sequelae and sensorineural hearing loss can be detected later, there is a need for a test to diagnose these cases; DNA detection in dried blood spots (Guthrie cards) has been described for this purpose with promising results. In this work we show our 5 year experience with DNA detection in Guthrie cards by a nested PCR technique, for late diagnosis of congenital CMV infection.

Results. The sensitivity and specificity of the nested-PCR, previously tested with cards from children with negative and positive viruria in the first three weeks of life, were respectively 93% and 100%. We tested thereafter 666 cards from children between 3 weeks and 12 years of age. CMV congenital infection was confirmed by this method in 64 (10%) of the cases. The main reasons for the search of CMV DNA in Guthrie cards were: Intrauterine growth retardation (1 positive card in 34 tested – 2.9% of positive cases) abnormal neurological findings in the child (24/219-11%), suspicion of maternal CMV infection during pregnancy (8/58-13.8%), hepatosplenomegaly, jaundice, elevation of liver enzymes in the newborn (1/19-5.2%). 55 cards from children with auditory impairments were also tested. CMV congenital infection was confirmed by this technique in 11 (20%) of the cases.

Discussion. The use of this technique allowed the diagnosis of CMV congenital infection in 10% of the studied cases. In our opinion, these results support the use of this technique for routine late diagnosis of congenital CMV infection, at an age when viral isolation is not able to do so. The results obtained with cards from children with auditory impairment are in agreement with previous publications that confirm CMV as a leading cause of sensorineural hearing loss.
B-38. Serology of CMV infection in fertile age women

R Bonamore,* E Mascheroni,* A Orlandi,* G Lunghi,* X Zhou,* S Gritti,*  
B Beltrami,* E Torresani,* C Boschetto**  

*Lab. Virologia, **Il Clin.Ost. Ginecologica, F. Policlinico Mangiagalli Regina Elena, Milano, Italy

Cytomegalovirus seroprevalence ranges between 30% and 50% with a serological rate of 1.6% in pregnancy in developed countries with high socio-economic level. Hence it is important to make an optimal serological definition for both fertile-age and pregnant women for whom the transmission of the infection to the fetus is possible; therefore accurate laboratory tests need to be performed in order to point out mainly the dangerous acute phase of the infection.

In this work we examined over the period 1.04.06-30.06.06 65 outpatients of our Hospital, pregnant or fertile-age women willing to become pregnant, whose results at the first screening test showed positive or ambiguous values for IgM antibodies to Cytomegalovirus (CMV Liaison DiaSorin). The serum samples have undergone the second IgM test (Vidas BioMerieux) and two different IgG Avidity tests produced by Biotest and DiaSorin. Finally 33 samples resulted positive or ambiguous for IgM with both methods, were tested for detection of CMV Dna by Real Time PCR (Bioline).

The results of the two Avidity tests were concordant in 49 cases: 6 samples with low avidity (i.e. the primary infection has been contracted within last 3 months), 8 with medium avidity and 35 with high avidity. 16 samples resulted conflicting. 5 of the 33 samples tested with Real Time PCR resulted positive showing CMV IgG Avidity values from 1 to 24%.

In conclusion we found that a simultaneous use of IgG Avidity test and DNA research improves the chronological definition of the acute phase of CMV primary infection.
Background/Objectives. Congenital CMV infection is a major cause of disability in newborns. One of the major sequelae of congenital CMV is sensorineural hearing loss (SNHL), occurring in up to 15% of congenital infections. Rapid, early detection of congenital CMV would provide the opportunity to consider antiviral therapies, could enable early habilitation and intervention, and might improve speech and language outcomes. Functional newborn hearing screening (NHS), now standard-of-care in the United States, does not provide information about potential etiologies of SNHL, and delays in definitive diagnosis impair the ability to intervene with specific therapies, including antivirals. These studies therefore sought to examine newborn blood spots (NBS) from an anonymized group of infants who had failed NHS for the presence of CMV DNA by real-time PCR, working toward a long-term goal of coupling newborn DBS CMV screening to all failed NHS tests.

Methods. Following Institutional Review Board approval, anonymous DBS were obtained in infants who had failed NHS, from the Minnesota Department of Health. DNA was extracted from three 3 mm blood spots using the X-tractor Gene™ system. For CMV PCR, Lightcycler® CMV UL54 primer/hybridization probes were utilized and PCR was performed on the Lightcycler® instrument using the following parameters: an initial 95°C for 10 minutes, then 45 cycles of denaturation at 95°C for 10s, annealing at 55°C for 15s, and elongation at 72°C for 15s. Melting curve analyses were performed and data acquired in the ‘continuous’ mode during an increase in temperature from 40oC to 80°C.

Results. The real-time PCR assay was highly sensitive, with a lower limit of detection of 2.5 genomes/reaction. Amplification of beta-globin from DNA extracted from DBS confirmed the adequacy of recovery of template. Of 87 children referred for further evaluation because of a failed NHS, 6 had CMV DNA identified in the NBS (7%). The mean viral load among positive samples was 813 genomes/microgram of DNA (SD, 574).

Conclusions. Real-time PCR is a sensitive technique for identification of CMV DNA from NBS. Examination of group of newborns who failed NHS suggests that approximately 7% of these infants have evidence of congenital CMV infection. NBS screening may be a useful and rapid adjunct to functional NHS and may enable more rapid etiologic diagnosis of SNHL in newborns.
Background. Abnormalities due to prenatal cytomegalovirus (CMV) are more commonly associated with primary than non-primary maternal infection during the first six months of pregnancy. Detection of IgM antibody can be long-lasting or non-specific. IgG antibody avidity increases gradually after primary infection and is a useful supplementary test for recent primary infection. When primary maternal infection is definite or doubtful, testing amniotic fluid (AF) at an appropriate stage of pregnancy further identifies those fetuses at risk of abnormalities, predominantly brain damage and deafness.

Methods/Results.
1. During 1998 to 2004, amniocentesis was performed at 15 to 25 weeks in 13 definite cases of primary maternal infection. Seven were clinical and six were subclinical. In three cases, CMV was identified in AF. Results of isolation and polymerase chain reaction tests were concordant. At delivery, CMV was identified in urine and/or saliva in three of 11 newborns tested. Results of amniocentesis and tests at delivery were concordant in 10 cases, and discordant in two cases. CMV was identified in 2 newborns for whom AF was negative at 15 and 20 weeks respectively. Two pregnancies were terminated: CMV was identified in one placenta tested. Permission for virology was declined for both fetuses.

2. During the same period, amniocentesis was performed at 14 to 23 weeks in 10 doubtful cases of primary maternal infection. Two were clinical and eight subclinical. In one case, CMV was identified in AF. CMV was identified in only this one of 10 newborns tested.

3. In six cases (seroconversion 2; IgM and rise in IgG 2; low or moderate IgG avidity 2) amniocentesis was negative, but testing of the newborns could not be confirmed.

Conclusions. It was diagnostically useful to test AF for CMV in selected cases, although the delay advised between diagnosis in the mother and the timing of amniocentesis was stressful for parents. In the setting of diagnostic private pathology, it is difficult to achieve appropriate testing at delivery.

Comments. The interpretation of avidity tests is not always definite in relation to the timing of infection. Results and interpretation vary between different methods, and may be modified by a manufacturer (eg, VIDAS). Results also vary between individuals, eg. not all past infections show high avidity; not all recent infections show low avidity; and some recent infections show persisting relatively low avidity.

References
B–47. CMV primary infections in pregnant women in Friuli Venezia Giulia region

G Dal Molin, P D’Agaro, P Burgrich, E Samar, C Biagi, F Petronio, C Campello

Dept of Public Med. Sc. University of Trieste, Lab. of Virology, IRCCS Burlo Garofolo Trieste, Italy

Objectives. To evaluate the outcome of primary CMV infections in pregnant women in relation to gestational age and prenatal diagnosis.

Materials and methods. 52 pregnant women attending the Centre for Prenatal Diagnosis of IRCCS Burlo Garofolo from 1996 to 2005 with diagnosis of CMV primary infection were included in the study. Some cases were sent to our centre from other laboratories of the Friuli Venezia Giulia region to confirm diagnosis. Serological analysis included IgG and IgM detection and IgG avidity determination. Biological samples including amniotic fluid were analysed by shell vial culture (SVC) and PCR (MIE and LA regions)

Results. The 52 primary infections were distributed fairly homogeneously during the study period with a mean of 4.7 cases/yr. The mean age of the enrolled pregnant women was 30.2 yrs (SD 4.68, median 29.4) and the 44% had an age between 25 and 29. Primary infection was recognized by seroconversion in 29 cases and by IgG avidity determination in 23 cases; seroconversion was more frequently detected after 2000 for a improvement in the CMV screening program. With respect to the gestational age, 33 infections were detected in the first 12 weeks, 5 cases between week 13 and 20 and 14 after the 20th week. All the infections after the 20th week were demonstrated by seroconversion while in the first 12 weeks the majority of infections (21/33) were recognized by IgG avidity. Amniocentesis was performed in 25 out of the 38 pregnant women infected in the first 20 wks and 13 resulted positive both in PCR and in SVC; 11 underwent voluntary abortion and 2 newborn were Infected and Symptomatic (SIN). One asymptomatic infected baby was born to a CMV amniotic fluid negative mother. Among the 13 women who refused the Amniocentesis: 5 underwent voluntary abortion, 3 delivered Non Infected Newborn, 3 Asymptomatic Infected Newborn and 1 an infected newborn with hepatitis. The overall mother-to-child transmission rate of the CMV infection was about 52%. The infected newborn were 40% and the SIN were the 29% out of the infected newborn.

Discussion. Our results underline the relevance of an integrated serological and virological approach to the correct diagnosis of CMV infections in pregnant women and the need of a skill analysis of the results for a correct dating of the infection. Moreover the relevance of the outcome of the primary CMV infections in pregnant women is emphasized by the 40% infection rate in the newborn and the 29% of SIN.
B-50. Epidemiological study of congenital CMV infection in newborns in Sweden

M-L Engman,1 G Malm,1 K Petersson,2 E Karltorp,4 M Forsgren,3 I Lewensohn-Fuchs2

1Divisions of Paediatrics, 2Clin. Virology, 3Obstetrics, 4ENT, Karolinska Institutet, Sweden

Background. Congenital cytomegalovirus (CMV) infection is the most common intrauterine infection and one of the most important causes of deafness in childhood as well as an important cause of neurological handicaps. The possibility to treat CMV infection has been limited due to toxicity of the drugs available. Development of drugs with improved therapeutic use for treatment and a safe vaccine for prevention is of high priority. The purpose of this study was to provide updated Swedish epidemiological baseline data to be able to make decisions regarding prophylaxis and antiviral therapy. In order to collect epidemiological data 6060 children born in Stockholm during one year were screened for the presence of CMV DNA.

Methods. Dried blood spot (DBS) filter papers were collected at the age of 3-5 days and screened for CMV DNA. A paediatrician examined positive cases and the diagnosis was confirmed by virus isolation in the urine. Children with confirmed congenital CMV infection were further evaluated by investigation of lever enzymes and head ultrasound. Hearing was assessed by otoacoustic emission (OAE) in the newborns and at follow up at 12-24 months. Maternal infection was categorised by analysing a serum sample collected in the first trimester and compared with a serum sample collected after delivery. In order to identify CMV DNA in DBS, a quantitative (TaqMan) PCR technique was used with primers and probe from the pol region. Extraction was performed by the use of MEM (Barbi et al., Clin Diagn Virol 1996;6:27-32). In addition 1000 serum samples from the mothers included in the study were determined for IgG antibodies against CMV by ELISA.

Results. We identified 12 children with CMV positive DBS and positive virus isolation in the urine. Seven cases were due to primary infection and 5 cases had a secondary infection. Only one child was symptomatic at birth and had a sensorineural hearing loss. However, 9 cases had few copies of CMV in the DBS but were negative in the urine. Of the mothers 70% were CMV IgG positive.

Conclusions. It is possible to use DBS in order to perform large scale CMV DNA screening in newborns. However, further virological and clinical follow up is necessary to evaluate the specificity and sensitivity of these newly developed methods. So far the Golden standard for identifying congenital CMV infection have been to isolate CMV from the urine of the child. With new methods it is possible that the Golden standard has to be revised.
B-51. Measurement of CMV IgG avidity improves the diagnosis of congenital cytomegalovirus infection

D Huzly, D Neumann-Haefelin

Department of Virology, Institute for Medical Microbiology and Hygiene, University Hospital Freiburg, Germany

Human Cytomegalovirus is the leading cause of congenital viral infection with an estimated incidence of 0.2% to 2.5% of life births. Only about 5% of congenitally infected children have CMV-related symptoms in the newborn period. Most children present at the age of 2–12 months, when acute primary CMV infection is common as well. No routine diagnostic method can differentiate between congenital, perinatal and later infection. CMV IgG avidity is used for the diagnosis of primary infection in adults, but it has never been assessed whether maturation to avidity is normal in congenitally infected children. We measured CMV IgG avidity in 31 children with suspected (n=25) or proven (n=6) congenital infection. The children were 2 weeks to 3.5 years old, when CMV infection was diagnosed. In all 6 children with proven congenital infection IgG avidity was high at the time of first diagnosis. IgM was negative in 5 of these cases. In 6/25 children with suspected congenital infection low avidity antibodies were measured. 5 of them had symptoms compatible with acute CMV infection, IgM was positive in 4 of these cases. In 1 case, IgG avidity of the mother was low as well and perinatal infection seemed probable. Avidity was high or moderate in the remaining 19 children. Symptoms were suspicious of congenital CMV infection in 15 of these children but follow up was not done. Determination of CMV IgG avidity is helpful for the diagnosis of congenital infection in combination with virus isolation from urine. The lack of CMV IgM does not exclude congenital or postnatal CMV infection.
B-54. Congenital cytomegalovirus infection: risk analysis via a neural analyzer

A Calvario,* L Carnimeo,* A Bozì,* C Birtolo,* ML Scarasciulli

*Laboratory of Virology - Hygiene, Epidemiology and Public Health, Policlinico Bari;
*Department of Electrical and Electronic Engineering, Politecnico Bari, Italy

Prevention and early diagnosis of CMV infected newborns are the only chance to reduce effects of sensorineural deficit strictly connected to congenital CMV infection.

These factors are both considerably influenced by a profound knowledge of viral natural history and by an increasing set of informations deriving from a strict management of mothers and newborns involved in CMV infection.

In these years, large literature on CMV congenital infection has been produced to improve the knowledge of this herpetic virus and, thanks to a multidisciplinary approach to this public health problem, quite a diagnostic/clinical progress has been achieved. Despite a growing amount of clinical data have been produced, unknown variables are still numerous.

The selection of fetal disease prognostic markers and adverse outcomes in newborns remains a major challenge for all those who attend to CMV infected babies’ management. Therefore, the knowledge of risk factors and the availability of fast accurate diagnostic tools which can enable clinicians to early identify infected newborns reveal fundamental.

On this proposal, a new engineering tool to support virologic diagnosis, called Neural Analyzer, is proposed with a double task: a correct detection of congenitally infected newborns and among these an exact indication of the most exposed ones to the risk of developing sensorineural sequelae. Starting from real data, the proposed Neural Analyzer can support early diagnosis of sensorineural deficit by identifying which children present a higher risk to develop it from a clinic/virological point of view.

Determinations will concern with the more interesting results in the CMV infection assessment both in mothers and in newborns; with regard to maternal determinations serological tests to define immunological status in pregravidic stage and in pregnancy will be analyzed together with prenatal diagnosis results in case performed in amniotic fluid samples by Real-Time PCR and cultural/immunofluorescence assay.

Concerning with neonatal determinations, virological issues, by cultural techniques in shell vial assay with HCMV IEA+IFMAb, on neonatal urine samples collected not later than 2 weeks of life will be reported; serum and PMNL sample collection data, tested by Real-time PCR, will be reported too.

Therefore the well-known critical aspect linked with CMV isolation by urine samples will be overcome through molecular DBS test results on neonatal Guthrie Cards, an essential diagnostic method for the early identification of congenital infected newborns.
POSTERS

Prognostic markers & Counselling (Session C)
C-05. Outcome of 10 children with congenital CMV infection in paediatrics at Besançon hospital

F Paratte,1 N Khayat,1 A Coaquette,2 L Dornier,1 C Guillermet,1 D Amsallem,1 A Menget,1 G Herbein1

1Paediatrics, 2Virology, ORL CHU Besançon-France

Introduction. Different prognostic factors for CMV congenital infection exist but are rarely available when symptomatic CMV infection occurs late after delivery. We present 10 recent cases of congenital infection with a wild range of symptoms and outcomes in order to determine the main data involved in prognostic.

Objectives. To determine which could be interesting prognostic markers and what could be long-term consequences of congenital CMV infection.

Methods. Ten children with congenital CMV infection (between 2 and 12 years-old) were analyzed. The infection was diagnosed by maternal serology during the pregnancy or by virus isolation in urines or saliva in the first 15 days of life or both. Their average age is 5 year-old.

Results. Five newborns had a severe CMV disease, 4 maternal primary infections, 1 recurrent infection. One died in neonatal period with a severe multivisceral infection. Three, with delayed diagnosis of non life threatening CMV infection, were not treated with Ganciclovir and had a poor prognosis with cerebral palsy and deafness. One, with prenatal diagnosis, received early Ganciclovir during 3 weeks. The result was satisfying except bilateral deafness and hepatitis. Five children had mild symptoms related to CMV, 2 maternal primary infections, 3 recurrent infections. 2 had digestive symptoms, 1 had a temporary neonatal thrombocytopenia, 2 had an neurologic disorders with favourable outcome. One of them received Ganciclovir during 2 weeks. The search for CMV viral DNA was also performed, in 2 different laboratories, by PCR with Dried Blood Spot from 8 of these children.

Conclusions. Maternal recurrent infection can give severe foetal infection as well as primary infection. Outcomes of CMV infection from our 10 patients were various. Microcephaly and foetal symptomatic CMV infection during pregnancy are 2 markers of poor prognosis. Nevertheless, antenatal diagnosis can be missed (7 out 10 in our work, including 3 severe diseases). For this reason CMV PCR with Dried Blood Spot seems to be actually the best way to diagnostic retrospectively congenital CMV infection. Moreover, it appears a lack of prognosis factors to help for therapeutic decision. We discuss the different ways to achieve an acceptable procedure of prevention of CMV congenital infection by the mean of analysis of different biological samples with serological and molecular methods.
C-15. Detection of cytomegalovirus (CMV) antibodies in urine and CMV congenital infection

A Coaquette, F Paratte, G Herbein, L Dornier, A Menget, C Guillermet, D Amsalle, N Khayat

Paediatrics, Virology, ENT, CHU Besançon, France

Background. CMV is a model of compartmentalized infection and immunity. Previous reports suggest that the detection of antibodies (Ab) against numerous virus in peripheric fluids, including urines, is related to an active viral infection.

Objective. To study the usefulness of anti-CMV Ab detection in urines for the diagnosis and prognosis of neonatal CMV infection.

Methods. From 2004 to 2006, a screening of CMV infection is performed with urines samples in the hospital of Besançon in 146 children (98 before 15 days of life, 48 between 2 weeks and 1 year-old). Patients with nephropathy are excluded. CMV is detected in MRC5 cells culture and an ELISA technique is used to detect anti-CMV IgG and IgM in undiluted urine. An immunofluorescence method allows the confirmation of positive results. Clinical data are collected.

Results. The rate of positive anti-CMV Ab in urine increases in children from 41.6% before 15 days of life to 63% between this term and the age of 1 year. We note a similarity between the previous 41.6% and the 45% of CMV seropositivity in pregnant women in our hospital. IgM are positive (+) in 7% out of the IgG+ samples and in 9.5% out of the IgG negative (-) samples. Infectious CMV is detected in 6% of the IgG+ samples but in 19% out of the IgG- samples. IgG are not detected in urine from infants born with a typical cytomegalic inclusion disease (CID) but are detected in 82% of lightly symptomatic newborns. Anti CMV-treatment leads to the apparition of anti-CMV IgG. Symptomatic infections (CID, microcephaly, cerebral palsy, delayed development, hepatitis, thrombopenia) are strongly linked to the detection of IgM and/or CMV in urine.

Conclusions. As previously described, detection of anti-CMV Ab in urine can be seen as a marker of probable CMV infection, not related to maternal Ab. Detection of IgG in urine is linked to a non progressive infection. Conversely, progressive CMV replication is linked to the decrease of IgG and/or presence of IgM in urine. Anti-CMV treatments were followed by the disappearance of IgM and appearance of IgG in urine. Larger studies are necessary to confirm the usefulness of Ab detection in urine for the diagnosis of this infection, as a complementary screening tool. It should be an accurate method for epidemiological studies in absence of blood samples and could be involved in the evaluation of the prognosis of the infection. This easy method can complete the choice of the diagnostic procedures.
C-35. Spontaneous improvement of fetal cytomegalovirus infection

B Tassis, M Furione*, A Quarenghi, C D'Amico, E Sipio, U Nicolini

Department of Obstetrics and Gynecology, Ospedale V. Buzzi, University of Milano *Department of Virology IRCCS Policlinico San Matteo, Pavia, Italy

Background. The patient, a 27-year-old primigravida, was referred to our Center for prenatal diagnosis, following primary human cytomegalovirus (HCMV) infection which had occurred at 12 weeks’ gestation.

Methods. Amniocentesis performed at 21 weeks’ gestation revealed the presence of virus both by the shell vial assay and PCR technique. According to our management protocol, the patient opted for fetal blood sampling, in order to investigate the degree of systemic infection.

Results. The fetal blood showed a low viral load (negative viremia, antigenemia 1/200.000 leukocytes, DNAemia 844 Geq/10 μL and low levels of HCMV-specific IgM (R=1.08), but elevated levels of transaminases (GOT =258 U/L; GPT=15 U/L) and GGT (349 U/L), high levels of serum beta2-microglobulin (9.6 mg/L), together with fetal anemia (Hb=9.3 g/dL), and thrombocytopenia (PLT=74x10³/microl).

Despite the evidence of fetal systemic involvement, the patient elected to continue the pregnancy and to monitor the fetal conditions. Serial ultrasound scans were always normal, as well as the MRI performed at 24 weeks’ gestation. Two subsequent blood samplings done at 24 and 28 weeks revealed amelioration of fetal anemia and thrombocytopenia, normal hepatic enzymes values, and decreasing beta2-microglobulin levels. The viral load remained low, while HCMV specific IgM slightly increased at 24 weeks’ gestation (R=2.6), and became negative at 28 weeks. The pregnancy progressed uneventfully until term. At birth, the newborn was viruric and a low level of viral DNA (10 Geq) was detected in blood together with iEmRNA, while virus-specific IgM were undetectable. The newborn infant was asymptomatic and is now thriving.

Conclusions. This case report underlines the unpredictable course of congenital HCMV infection, where spontaneous resolution of systemic damage may occur without any intervention.
C-39. Risk factors of post-natal symptomatic CMV infection in preterm infants

L Gabrielli,* MG Capretti,* T Lazzarotto,* M Lanari,* S Pignatelli,*
P Dal Monte,* G Faldella,* MP Landini*

*Dept. of Clinical and Experimental Medicine, Sect. of Microbiology, *Dept. of Preventive Pediatrics and Neonatology, St. Orsola Malpighi GH, University of Bologna, Italy

Background. Postnatal human cytomegalovirus (CMV) infection is usually asymptomatic in term babies, while preterm infants are more susceptible to symptomatic CMV infection. Breastfeeding plays a dominant role in the epidemiology of transmission of postnatal CMV infection, but the risk factors of symptomatic CMV infection in preterm infants are unknown.

Methods. The study population consisted of 57 preterm infants (gestational age of <32 complete weeks) and their 52 mothers. During the first week, postpartum maternal blood was examined by serology for CMV and weekly urine samples from infants were processed for virus culture until discharge of the neonate. In addition, samples of fresh breastmilk were processed weekly for virus culture. A genetic analysis of virus variant was performed.

Results. 35 mothers (67%) were positive for CMV IgG antibodies and 17 (33%) were seronegative. In the seronegative group, CMV isolation in breastmilk was negative during the study period and none of the 17 infants developed viruria, whereas in 15 (43%) of the 35 seropositive mothers the virus was isolated from breastmilk. Only one (4.5%) of 22 infants born to 20 virolactia negative mothers had a CMV infection and he showed mild neutropenia. An infectious virus was detectable in urine in 7 (39%) of 18 preterm infants born to 15 virolactia positive mothers; 3 infants had asymptomatic CMV infection, 2 developed moderate neutropenia and 2 showed a sepsis-like illness with tachycardia, tachypnea and repeated desaturation spells. No long-term sequelae linked to CMV infection were present in infected babies at 6 and 12 months’ follow-up. In a case of preterm twins who showed a different pattern of CMV disease, even though exposed to the same infective milk, viral genotype was studied. Samples from the mother and sons were analysed and a different gN genotype was repeatedly detected in each twin (the one with sepsis-like illness was gN-4c/gB-2 while the other with moderate neutropenia was gN-1/gB-2), while the mother’s milk showed an alternate or concomitant shedding of CMV variants (gN-4c/gB-2 and gN-1/gB-2). Genetic analysis of any virus variant in the other cases are in progress.

Conclusions. In our experience, important factors in determining the occurrence and severity of postnatal CMV disease in preterm infants are virolactia and the different virulence of any gN variant. According to literature studies, gN-1 strains seems to be less virulent than the gN-4 group.
C-40. Neuroimaging findings and neurological outcome in symptomatic congenital cytomegalovirus infection

EM Ruga, I Balao, R Manara, R d’Elia, P Drigo
Department of Pediatrics, University of Padova, Padova, Italy

Background/Objectives. Congenital cytomegalovirus (CMV) infection can cause encephalopathy with cognitive, motor, audiological or visual impairments. We evaluated the correlation between the neuroimaging findings (brain CT and MRI) and the neurodevelopmental outcome in children with symptomatic congenital CMV infection.

Methods. The psychomotor development steps and the neurological sequel of 12 children with symptomatic congenital CMV infection, followed at our Department between 1994 and 2006, were evaluated reviewing medical records. Nine patients had at least one CT scan within 3 months of age and 10 had at least one MRI. The mean follow-up was 53 months (range 13-144 months). CT findings were graduated according to a scoring system recently reported in the literature (Noyola et al., J Pediatr 2001).

Results. CT examination was abnormal in 7 out of 9 children showing cerebral calcifications (6/9), ventriculomegaly (6/9), periventricular cysts (4/9) and cerebellar hypoplasia (4/9). Noyola’s CT score was ≥3 in six patients and =0 in one. All the patients with CT score =3 had abnormal development and neurological sequel: 6 cerebral palsy, 5 epilepsy, 5 sensorineural hearing loss (SNHL) and 3 corioretinitis. The patient with CT score =0 for single periventricular cyst showed SNHL. In 2 of 9 patients the CT scan demonstrated absence of abnormalities: 1 patient had good neurodevelopmental outcome and SNHL. Six out of 12 children had microcephaly at birth: CT examination showed multiple calcifications in all of them. MRI showed white matter changes (10/10) with cerebellar hypoplasia (3/10), cortical malformations (3/10), periventricular cysts (5/10) and hippocampal dysplasia (4/10). Children with cerebellar hypoplasia (3) had cerebral palsy and epilepsy and 2 of them respectively showed SNHL and corioretinitis. Three children with cortical malformations had the same pattern of neurological sequel. Three of 10 patients had only white matter changes: all showed good neurodevelopmental outcome and in 1 SNHL was observed.

Conclusions. Our results show that in congenital symptomatic CMV infection microcephaly and CT scan abnormalities are associated with mental retardation and motor deficits, as previously reported in literature; the absence of microcephaly and CT abnormalities have a good prognostic value apart from SNHL. Moreover, our results might suggest that isolated white matter changes detected by MRI are not associated with poor neurological outcome.
C-42. HCMV gN genotypes distribution among congenitally infected newborns monitored during a one-year follow-up

S Pignatelli,* M Lanari,* P Dal Monte,* L Gabrielli,* T Lazzarotto,* G Rossini,* B Guerra,* MP Landini*

Departments of *Clin & Experim Med, Sect. of Microbiology, *Preventive Pediatrics and Neonatology, *Obstetrics and Gynecology, St. Orsola Malpighi GH, University of Bologna

**Background.** Human cytomegalovirus (HCMV) is one of the most important pathogen which can cause congenital infection with a wide spectrum of clinical manifestations differing in terms of severity at birth and sequelae. Little is known about the role of the genomic and phenotypic peculiarity of each HCMV clinical isolate in contributing to severity and progression of infection. The study of genetic polymorphisms among HCMV wild-type strains has shown that some genes codifying for functions essential to viral replication can affect their pathogenicity. The polymorphic locus UL73, encoding for the envelope glycoprotein gN is one of them. Among HCMV clinical isolates UL73 shows 7 genomic variants (gN-1,gN-2,gN-3a,gN-3b,gN-4a,gN-4b,gN-4c) that were associated with different virulence of strains. Aim of this work is to analyse the gN genotypes distribution and their potential pathogenicity among newborns infected in utero.

**Methods.** Viral DNA extraction was performed using QIAGEN Biosprint15 on urine from 100 newborns. UL73 was amplified from viral genome by nested PCR, as previously reported (Pignatelli et al., Transfusion 2006) and genotyped by sequencing. The relation between gN genotypes and neonatal clinical parameters, such as symptomatology at birth (87 cases), sequelae during a one-year follow-up (52 cases) or virological determinations such qPCR on maternal amniotic fluid or neonatal urine and antigenemia (work in progress) was analysed using contingency tables and chi-square test or Fisher–exact test for cells of small size.

**Results.** Data from this study confirmed that gN-1 carrying strains significantly associate to milder clinical manifestations, in respect to gN-4 and gN-3 groups. In particular, a statistically significant (p<0.05) different distribution of gN genotypes arise from the following comparisons: a) considering symptomatology at birth, gN-1 associates to symptomatic infections (SI) more rarely than gN-4a and gN-3a (gN-1 SI 9/21 vs gN-3a SI 10/11, gN-4a SI 17/19, gN-4b SI 14/18); b) taking into account the presence of long-term sequelae, gN-4b associates with adverse sequelae (STA) more frequently than gN-1 (gN-1 STA 3/12 vs gN-4b STA 7/11). Preliminary results on quantitative parameters of viral infection also show that isolates belonging to gN-4 group relate with higher values of viral DNA load than gN-1 variants both in amniotic fluid and newborns’ urine.

**Conclusions.** Our findings suggest that the evaluation of gN genotype from the infecting strain could be an useful support to monitor HCMV congenital infection and may provide informations about progression and prognosis of infection, obviously in association with the usual clinical parameters.
POSTERS

Immune response (Session D)
**Background.** Cytomegalovirus (CMV) is the most common cause of congenital infection worldwide and occurs as a result of transplacental transmission of the virus. The human neonate is highly susceptible to infection due to a combination of immaturity of the immune system and antigenic inexperience.

**Objectives.** This study uses the *in vivo* model of congenital CMV to examine both the humoral and cell mediated immune responses, in vertically infected neonates and their mothers.

**Methods.** Ten pairs of matched neonates and their mothers were evaluated for specific IgM responses to 3 immunodominant CMV antigens; pp38 (pUL80a), pp52 (pUL44) and pp150 (pUL32). T cell mediated immunity was assessed by measuring cytokines using a multiplex microarray assay.

**Results.** In contrast to conventional EIA testing for CMV specific IgM which found 5 of the mothers and 4 of the neonates to be positive, western immunoblotting showed all 10 adults and 9 newborns to be positive. Eight mothers and 9 newborns had serological evidence of primary infection. All neonates showed a response to pp38, an assembly protein, 9 responded to the pp52 immediate early antigen but only 4 had reactivity to the pp150 tegument associated protein. Of the mothers, 8 had pp38 reactivity, 10 showed a response to the pp52 antigen and 7 to the pp150 antigen. Levels of IFN-γ were high in both groups (mean ± SEM: neonates= 657±238 pg/mL, mothers= 1072±677 pg/mL, pNS), however neonates had significantly higher levels of IL-8 (316±136 pg/mL vs 48±28 pg/mL, p<0.005). Similar levels of IL-2, IL-7, IL-10 and IL-12 were measured in both groups, but levels of IL-1α, IL-1β, IL-4, IL-6 and TNF-α were either absent or low.

**Conclusions.** In response to CMV, neonates and adults mount a predominant Th1 response as evidenced by the presence of IL-2, IL-8, IL-12 and IFN-γ with concomitant lack of IL-4. These findings suggest that the neonate when presented with infection in utero is capable of mounting an individual response, however the lower IFN-γ and higher IL-8 levels suggests reduced immune responsiveness when compared to their adult counterparts.
POSTERS

Prevention (Session E)
E-27. Congenital cytomegalovirus infection: long-term outcome

L Pugni, S Binda*, MC Casciati, A Proto, S Montella, M Barbi*, F Mosca

NICU, Cl. L. Mangiagalli, University of Milan, *Institute of Virology, University of Milan, Italy

Background. CMV is the leading cause of congenital infection in humans (0.2–2.5% of all live-births); 5–10% of congenitally infected infants are symptomatic at birth (mortality 10–30%, significant sequelae in 90% or more of survivors). Asymptomatic congenitally infected infants usually develop normally, but 10–15% of them develop sequelae, particularly sensorineural hearing loss (SNHL). Ganciclovir represents the treatment of choice for symptomatic infection.

Objectives. 1) To evaluate retrospectively the incidence of sequelae in newborns with congenital CMV infection 2) To determine the ability of clinical/virologic findings to predict long-term outcome

Methods. The study population included all infants with congenital CMV infection born at our Hospital between 2002 and 2005. Exclusion criteria: newborns born of HIV infected women. Dried blood spots (DBS) collected on filter paper (Guthrie card) in the first days of life were tested for CMV by PCR; congenital infection was confirmed by isolation of CMV from urine. Congenitally infected infants were included in a follow-up study: virologic tests, clinical, psychometric, ophthalmologic and audiologic evaluations were performed at birth, at 3–6–12–18 months of age and annually thereafter until age 6 years.

Results. During the study period 32 newborns (mean GA = 37.6 wks, mean BW = 2516 g) were diagnosed as congenitally infected; 15 (46.8%) of them were born of women with primary infection during pregnancy. 9/32 (28.1%) infants were symptomatic at birth (jaundice 2 cases, thrombocytopenia 2, intrauterine growth retardation 1, chorioretinitis 1, CNS abnormalities 3) and 8 of them received intravenous ganciclovir treatment. Five (55.5%) of the 9 babies symptomatic at birth showed symptoms at follow-up evaluation (SNHL 2 cases, cerebral palsy 2, psychomotor retardation 1), while two (8.6%) of the 23 babies asymptomatic at birth developed long-term sequelae (SNHL 1 case, hepatitis 1). There was no significant difference in the duration of viral excretion into saliva and urine between children with and without symptoms.

Conclusions. Our findings confirm the high incidence of sequelae in congenitally infected infants, both symptomatic and asymptomatic at birth. Since long-term sequelae caused by congenital CMV infection occur frequently, universal newborn screening for CMV, by using DBS test, could be recommended to detect sequelae as early as possible, so that infants can receive intervention promptly.
E-33. Molecular epidemiology of human cytomegalovirus (HCMV) in families of pregnant women with primary HCMV infection

G Campanini, M Zavattoni, M Furione, E Percivalle, MG Revello, G Gerna
Servizio di Virologia, IRCCS Policlinico S Matteo, Pavia, Italy

Objective. To investigate potential source of infection for pregnant women with primary HCMV infection.

Methods. 27 pregnant women (7 primiparous) with primary HCMV infection and their households were investigated. Pregnant women, husbands, and children living in the house were examined for HCMV in amniotic fluid, saliva and/or urine by conventional and/or rapid (shell vial) isolation techniques. The highly polymorphic UL 146 gene sequence was compared among HCMV strains of the same family group to identify a common source of infection.

Results. Overall, HCMV was detected in 22/27 (81.5%) pregnant women, 13/20 (65%) children (aged 9–95 months, median 29.5) and 3/21 (14.3%) participating husbands. Primary HCMV infection was diagnosed in the 3 HCMV excreting husbands. HCMV was concomitantly recovered in the pregnant woman and in one or more households in 11 cases. In particular, HCMV was detected in the pregnant woman and in one or more children in 8 cases, whereas in 3 cases the woman, the husband and one child were all excreting HCMV at the same time. Preliminary results obtained from 8/11 families indicate complete UL 146 sequence homology among HCMV strains recovered from members of the same family. HCMV congenital infection was diagnosed in 7/12 newborns examined so far.

Conclusions. Preliminary results confirm that young children living in the house do represent a major source of infection for seronegative pregnant women (and households in general). HCMV seronegative pregnant women should be aware of the problems related to HCMV infection and should be informed about hygienic measures to be taken to reduce the risk of acquiring primary HCMV infection during pregnancy.
POSTERS

Therapy (Session F)
F-12. Congenital cytomegalovirus infection in preterm newborn: case report

G Corona, S Carcione, N Decembrino, L Barbuscia, V Cordaro, I Barberi

Department of Pediatric Sciences, Neonatal Intensive Care Unit, University of Messina, Italy

Introduction. Cytomegalovirus (CMV) is a ubiquitous human virus. More than 90% of primary infections are asymptomatic in healthy adults and children but it may cause severe disease and sequelae in newborns after prenatal transmission. Intrauterine primary CMV infections are second only to Down’s syndrome as a known cause of mental retardation.

Clinical case. A male infant was born after 31 weeks of gestation because the pregnancy was complicated by oligohydramnios and preterm premature rupture of membranes. In the first trimester of pregnancy the mother had anti CMV-IgG. The baby showed petechiae-purpura on left arm and right thigh, lethargy, hypotonia, thrombocytopenia, jaundice, elevated ALT and haemolysis. The laboratory test revealed positive results for CMV IgG and IgM antibodies (IgM 45.50AU/mL, IgG 28.30 IU/mL) and avidity test. Quantitative assessment of the CMV DNA load was performed in blood and urine samples (blood CMV-DNA =573 copies/mL; urine CMV-DNA=930000 copies/mL). The baby was treated with ganciclovir (12 mg/kg – 2 times/day for 6 weeks) and during the treatment blood and urine viral load was determined by quantitative Real Time PCR. The ganciclovir therapy was associated with a reduction in blood and urine viral load and completely normal blood and platelet count. Infected baby was followed up at 3, 6, 12 months of life, and then annually until the 6 years according to a protocol of NICU at the University of Messina. Follow up included physical, neurological evaluation, cranial ultrasound and/or and computed tomography scan of the brain, abdomen ultrasound examination; neurodevelopmental evaluation; auditory brainstem responses; fundus oculi; blood sampling for laboratory tests. During the first year of life negative results for IgM anti CMV and DNAemia were obtained. The viral urine load showed a progressive but not completed reduction.

Conclusions. Because there is currently no treatment during the pregnancy, our case demonstrates how an early and corrected diagnosis of congenital CMV symptomatic infection is very important to modulate an antiviral treatment and to minimize long-term sequelae.

In our case, the baby showed clinical signs of congenital CMV infection, as thrombocytopenia, jaundice, anaemia, hepatosplenomegaly and hypotonia. Now the baby is 20 months and the follow-up showed only a mild neurodevelopment retardation.
F-13 Congenital cytomegalovirus infection: therapy and follow-up in newborns

G Corona, F Borruto, N Decembrino, S Carcione, MS Leonardi,* I Barberi

Dep. of Pediatric Sciences, NICU, *Virology, University of Messina, Italy

Introduction. Congenital cytomegalovirus (CMV) infection is the leading cause of congenital virus infection in developed countries, occurring in 0.3% to 2.4% of all live births depending on the sero-prevalence of the population examined. In Italy the prevalence of antibodies to CMV in adults ranges from 70% to 80% and congenital infection occurs in about 0.2-2/100 births.

Congenitally infected infants are symptomatic at birth in about 10% to 15% of cases.

Objective. The congenitally infected newborns are followed up from birth at NICU of the University of Messina to evaluate the incidence of infection and development of neurologic sequelae at follow up.

Patients and Methods. In the years 2001–2005, we studied 1350 newborns at high risk of congenital CMV infection retrospectively.

Results. 13 (0.96%) newborns with congenital CMV infection were identified and 9 of them (69.2%) were preterm (G.A. 30.5±4.5 weeks). The detection of viral DNA was positive in 6 cases (46.2%). Of 13 newborns enrolled, 7 had symptoms: post-haemorragic hydrocephalous (1 case: 7.7%); respiratory distress (3 cases: 23%); prematurity and low birth weight (9 cases: 69.2%). The cranial ultrasound examination demonstrated thalamic calcifications in 3 newborns (23%). Six children with positive serological results were treated with ganciclovir 15 mg/kg/day i.v. for 15 days and 10 mg/kg/day i.v. three times a week for 3 months. During the treatment the infected newborns were followed with sequential laboratory evaluation including complete blood count, platelet count, liver transaminase levels, bilirubin levels controls. No significant collateral toxic effects due to the therapy were identified. Finally, two newborns were treated with ganciclovir 12 mg/kg/day i.v. for 6 weeks, following the recent indications reported in literature. Newborns were followed up at birth and 3–6–12-24–36 months of life. In the first year of life blood samples were examined for CMV-IgM test and CMV-DNA by PCR. One newborn (7.7%) developed severe neurologic deficits. No treatment was performed in newborns with asymptomatic CMV infection and none developed sequelae during the follow up.

Conclusions. The corrected diagnosis of congenital CMV infection is the first step for an effective and early treatment. In Italy the CMV screening during the pregnancy is not mandatory but the increasing attention to the problem improves the possibility of early diagnosis.
F-52 Effects of ganciclovir therapy on selected hematological parameters in treatment of congenital cytomegalovirus infection in newborns

M Golikowska, A Dobrzafksa, D Gruszfeld
The Children’s Memorial Health Institute, NICU, Warsaw, Poland

Objective. Evaluation of the effects of ganciclovir (GCV) therapy in symptomatic congenital cytomegalovirus (CMV) infection on hematological parameters in newborns.

Methods. Neonates with symptomatic congenital CMV infection assigned to receive treatment with intravenous GCV. During and after GCV treatment complete blood count was performed at baseline, during treatment (at 7th, 14th and 21st day) and post treatment. The individual doses of GCV were used and its blood levels were analyzed (with HPLC - high performance liquid chromatography) at 2 different time points (0h and 4.5h after the end of GCV infusion). Individual GCV doses and duration of treatment depended on severity of the illness and GCV concentration.

Results. A total of 40 newborns with a history of symptomatic congenital CMV infection, hospitalized at NICU between January 2002 and December 2004, treated with GCV were included in the study. The mean duration of therapy was 20 days. The mean daily dose of GCV was 11.1±5.1 mg/kg/day. The mean serum concentration was 6.62±1.49 mcg/mL and 1.91±0.81 mcg/mL at 0h and 4.5h after the end of GCV infusion respectively. The following side effects were noted: neutropenia in 11 (27.5%) cases, anemia in 13 (32.5%) newborns. No thrombocytopenia was observed as the effect of GCV treatment. Several laboratory parameters (neutrophils count, hemoglobin concentration, reticulocyteosis and platelet count) were analyzed to determine their correlation with duration of treatment with GCV and/or its dose. Treatment with GCV decreased neutrophils count in all groups (with pre treatment neutropenia and with normal neutrophils count), but it was statistically significant (p<0.001) only in the group with initially normal neutrophils count. There was no correlation between duration of therapy and neutrophils count. Treatment with GCV increased platelet count (p<0.001), but only in patients with pre-treatment thrombocytopenia. Treatment with GCV decreased hemoglobin concentration in all patient groups with or without pre-treatment anemia (p<0.001). There was no correlation between dose of GCV (neither duration of therapy) and platelet count or hemoglobin concentration.

Conclusions. During short time observation period GCV was well tolerated in newborns if serum concentration monitoring and the individual doses were used. The most frequent side effects were neutropenia, which correlated with the dose of GCV used (but not the duration of treatment) and anemia. Improvement of some laboratory parameters during treatment was observed in selected group of patients (with initial thrombocytopenia).
F-53 Long term treatment with oral valganciclovir for congenital cytomegalovirus (CMV) infection

A Méndez-Echevarría,¹ F Baquero,¹ MJ García,¹ M de José,¹ F del Castillo,¹ M Brunet²
¹Hospital La Paz, Madrid; ²Hospital Clínic, Barcelona, Spain

**Backgrounds.** Therapy with intravenous ganciclovir during 6 weeks can prevent hearing loss in patients with congenital CMV infection. Pharmacokinetic studies in infants have reported a peak concentration (Cmax) of 7 µg/mL following intravenous doses of 6 mg/kg. Up to 21% of children treated with intravenous ganciclovir will develop hearing loss within 1 year after therapy. Long term valganciclovir treatment may obtain better results. However there are no studies reporting the appropriate dose in neonates.

**Methods.** We describe two cases of congenital CMV infection with central nervous system involvement that received compassionate therapy with oral valganciclovir. The plasma trough (Cmin) and peak concentration (Cmax) were determined by high-performance liquid chromatography.

**Results.** Both cases were treated with intravenous ganciclovir during 6 weeks, followed by oral valganciclovir during 6 months. CASE 1. The initial dose of valganciclovir was 15 mg/kg/day, obtaining no detectable Cmin and a Cmax of 3 µg/mL after 1 month of treatment. This dose was switched to 500 mg/m²/day in 1 dose, obtaining a Cmin of 0.25 µg/mL and a Cmax of 8.9 µg/mL after 1 month of treatment. Urine culture and CMV antigenemia remained negative in following controls and no hearing loss deterioration was detected. CASE 2. The dose of valganciclovir used was 500 mg/m²/day in 2 doses, obtaining a Cmin of 1.1 µg/mL and a Cmax of 7.9 µg/mL after 1 month of treatment. Urine culture remained negative in following controls and no hearing loss deterioration was detected.

**Conclusions.** Valganciclovir 500 mg/ m²/day reached similar plasma levels than intravenous ganciclovir in our two patients. Long term therapy with this drug was well tolerated and no significant adverse effects were observed. In both cases, no hearing loss deterioration was observed.

<table>
<thead>
<tr>
<th>CASE</th>
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<td>Signs at birth</td>
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<td>Intracranial calcifications, Hepatosplenomegaly.</td>
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<td>Diagnostic tests</td>
<td>(+) Urine culture for CMV (+) Antigenemia assay</td>
<td>(+) Urine culture for CMV (+) PCR in blood sample</td>
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<td>First Hearing Assessment (at birth)</td>
<td>Bilateral mild loss (20 and 40 dB)</td>
<td>Bilateral mild loss (20 dB)</td>
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<tr>
<td>Last Hearing Assessment (6 months)</td>
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<td>Bilateral mild loss (20 dB)</td>
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<tr>
<td>Outcome (6 months)</td>
<td>Normal</td>
<td>Mild hypotony and development delay</td>
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F-57. Congenital Cytomegalovirus (CMV) infection leading to neonatal cholestasis and response to ganciclovir

I Shah, S Bhatnagar

Department of Pediatric Hepatobiliary Clinic, B.J. Wadia Hospital for Children, Parel, Mumbai, India

Congenital CMV is known to cause neonatal hepatitis with varied outcome. We present 6 children who had congenital CMV induced neonatal hepatitis and their response to treatment with ganciclovir.

Conclusion. Congenital CMV infection can lead to either a fulminant course or a chronic liver disease or recover completely. Ganciclovir may have a useful role if given early in the course of the disease before severe liver cell damage occurs.

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<th>Patient Details</th>
<th>Patient 1</th>
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<th>Patient 4</th>
<th>Patient 5</th>
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<td>2 ½ months</td>
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<td>4 days</td>
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<td>Since birth</td>
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