

210 - Session A.01: Epidemiology & Burden of Disease

Date: 01/05/2017

Time: 08:30 - 10:25 hrs

Keynote lecture

A-KEY-001

Is cytomegalovirus really an important pathogen?

Paul Griffiths

University College London, Centre for Virology, LONDON, United Kingdom

One way of approaching this question is to calculate the number of patients who suffer annually from CMV infection. An alternative way is to ask whether CMV causes more or fewer cases than other infections in the same patient population. For example, among congenital infections, CMV is the most common and also damages more babies than does rubella. In transplant patients, CMV is the most common opportunistic infection and causes more disease than any other virus. In AIDS patients, it remains a significant contributor to mortality, even though its contribution is less likely to be revealed nowadays by overt end organ disease such as retinitis. In non-immunocompromised patients admitted to intensive care, CMV frequently reactivates and is associated with increased ventilator-dependence and length of hospital stay. In the elderly, CMV is associated with immunosenescence as the immune system progressively commits more and more resources to controlling this infection. Finally, in the general population, CMV is associated with excess mortality whose magnitude is equivalent to raised levels of biomarkers such as C-reactive protein. Thus, taken overall, I conclude that CMV is an important pathogen whose disease associations are poorly recognised. It follows that control of CMV at the population level by means of immunisation would likely produce greater medical benefits than are generally realised.

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Date: 01/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

A-OP-001

Community Engagement to Improve Prevention and Treatment of Congenital Cytomegalovirus Infection

Laura Gibson¹, Kristen Spytek², Timothy Kowalik¹

¹University of Massachusetts Medical School, Departments of Medicine and Pediatrics, WORCESTER, MA, United States of America

²National CMV Foundation, TAMPA, FL, United States of America

In recent years, a community of parents, researchers, and others dedicated to advancement in the field of congenital CMV infection (cCMV) has increased in number and breadth. To harness their collective expertise and passion, a partnership among these stakeholders is being developed. The primary goal of the partnership is to create a global network to link resources and provide services that support collaboration and accelerate progress in cCMV awareness, prevention, and treatment.

This process began with the Congenital CMV Community Engagement Forum at the CMV Public Health & Policy Conference in September 2016. The purpose of the forum was to begin developing a partnership among diverse stakeholders, and designing a cCMV network. Given the creative nature of the activities, the meeting followed the “charrette” model of human-centered design, an engagement approach often used in architecture that focuses on solutions to a design challenge impacting all members of a community. A team of architects with expertise in the charrette model facilitated the session at their office. Working in small groups, forum attendees explored features that might benefit or impair a cCMV network, and concepts such as communication, inclusion, access, content, and operations. Building on ideas generated at the forum, a development team has been created to continue this design process and create some basic components of the partnership before seeking feedback from the larger cCMV community. These activities are being funded by a Tier I grant from the Patient-

Centered Outcomes Research Institute (PCORI). Through sequential tiered awards, PCORI resources and support provide a framework to guide the partnership development process, especially during the early stages.

Ongoing activities to build a broad partnership of stakeholders and a global cCMV network will be described. Led by affected families, the field of congenital CMV infection stands to benefit greatly from this collaborative process.

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Date: 01/05/2017

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BOTH ORAL & POSTER PRESENTATION

A-OP-002

Cost of illness of congenital CMV infection

Marjolein Korndewal¹, Marlies Weltevrede², M. Elske Van den Akker - van Marle¹, Anne Marie Oudesluys - Murphy¹, Hester De Melker², Ann Vossen¹

¹Leiden University Medical Center, Leiden, Netherlands

²National Institute for Public Health and the Environment, Center for Infectious Diseases, Epidemio, BILTHOVEN, Netherlands

Background

Congenital cytomegalovirus infection (cCMV) is the most common congenital infection worldwide. It can cause symptoms at birth as well as long-term impairment, including sensorineural hearing loss and developmental delay. This study estimates cCMV-related healthcare costs in the Netherlands in the first six years of life.

Methods

In a nation-wide retrospective cohort study 156 children with cCMV were identified by testing 31,484 stored neonatal dried blood spots using polymerase chain reaction. Use of healthcare resources in the first six years of life by children with cCMV and a matched cCMV-negative control group was analyzed. The average total healthcare costs per child were calculated from a healthcare perspective, by multiplying healthcare resource use by its reference prices.

Results

Medical data of 133 cCMV-positive and 274 cCMV-negative children were analyzed. Mean healthcare costs of children with cCMV (€6,113) were higher than those of cCMV-negative children (€3,570). The additional healthcare costs of cCMV were €2,544 (95% CI -€451, €5,538) per child in the first six years of life. When cCMV-positive children had symptoms at birth costs (€15,922) were more than four times higher than in asymptomatic cCMV-positive children (€3,730). In the Netherlands a total of €2.3 million could be attributed to cCMV for the whole birth cohort (184,634 children) of 2008 in the first six years of life.

Conclusions

Children with cCMV, especially those symptomatic at birth, accrue higher healthcare costs than cCMV-negative children in the first six years of life, although this is not statistically significant. Costs of special needs education, psychological healthcare and non-healthcare costs such as productivity losses of parents will further increase costs. In addition to the health impact, the economic impact needs to be taken into account in preventive measures aiming to reduce the prevalence and burden of cCMV.

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BOTH ORAL & POSTER PRESENTATION

A-OP-003

First outcomes of an international project on a universal neonatal screening for congenital CMV infections

Katrin Neumann¹, Khalid Shahada², Klaus Überla³, Thomas Lücke¹, Norbert Teig¹, Stefan Dazert¹, Stefan Volkenstein¹, Hans-Joachim Trampisch¹, Emmanouela Dimitrakopoulou¹, Peter Kern¹
¹Ruhr University Bochum, Dept. of Phoniatics and Pediatric Audiology, BOCHUM, Germany
²Hamad Medical Corporation, Audiology and Balance Center, DOHA, Qatar
³University Erlangen, Inst. of Clinical and Molecular Virology, ERLANGEN, Germany

An International Congenital CMV Consensus Recommendations Group recently provided recommendations for an early prevention, identification, and intervention of congenital CMV (cCMV) infections based on a systematic literature review (Rawlinson et al. 2016). Two screening strategies were documented—one aimed at the detection of maternal CMV infections by testing pregnant women, the other one was a universal screening of all neonates, based on real-time PCR of saliva. The latter method was recommended as to be considered. However, it was stated that more data are needed including cost-benefit analyses. A recent cost-benefit analysis has proven a universal neonatal screening as cost-effective (Gantt et al. 2016). This international project running in Germany and Qatar examines the feasibility and cost-effectiveness of a universal neonatal screening for cCMV infections. 12,000 neonates are planned to undergo a CMV-screening by real-time PCR-based testing of liquid-saliva specimens. If a cCMV-infection is confirmed, babies are enrolled in a 6-year audiological, neurological, radiological, and ophthalmological follow-up program.

By the end of 2016, CMV-screenings (specificity 99.7%, sensitivity 100%) of 3242 neonates from Germany and 1687 neonates from Qatar identified cCMV-infections of 14 babies in Germany and of 4 babies in Qatar, corresponding to a prevalence of 0.4 and 0.2 %, respectively. Five of the initially asymptomatic children developed symptoms during their first year of life. The high prevalence, the considerable proportion of initially asymptomatic infants who manifested sequelae later on, and the high test validity of the saliva testing support a strategy of universal rather than targeted maternal screening for cCMV infections.

Rawlinson WD et al. (2016). Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. Personal communication, Oct. 25, 2016.

https://www.ncbi.nlm.nih.gov/pubmed/?term=Gantt%20S%5BAuthor%5D&cauthor=true&cauthor_uid=27723885 et al. Cost-effectiveness of Universal and Targeted Newborn Screening for Congenital Cytomegalovirus Infection. <https://www.ncbi.nlm.nih.gov/pubmed/27723885> 2016 Dec 1;170(12):1173-1180.

*Funded by QRNF, NPRP 7-1845-3-480

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Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

A-OP-004

Characteristics and Hearing Outcomes of Symptomatic Congenital CMV Infection: Findings from the CHIMES Study

Shannon Ross¹, April Palmer², Amina Ahmed³, Marian Michaels⁴, David Bernstein⁵, Pablo Sanchez⁶, Kristina Feja⁷, Audra Stewart⁸, Karen Fowler¹, Suresh Boppana¹

¹The University of Alabama at Birmingham, Pediatrics, BIRMINGHAM, United States of America

²University of Mississippi Medical Center, Pediatrics, JACKSON, United States of America

³Carolinas Medical Center, Pediatrics, CHARLOTTE, United States of America

⁴University of Pittsburgh, Pediatrics, PITTSBURGH, United States of America

⁵Cincinnati Children's Hospital Medical Center and University of Cincinnati, Pediatrics, CINCINNATI, United States of America

⁶Ohio State University-Nationwide Children's Hospital, Pediatrics, COLUMBUS, United States of America

⁷Saint Peter's University Hospital, Pediatrics, NEW BRUNSWICK, United States of America

⁸University of Texas Southwestern Medical Center, Pediatrics, DALLAS, United States of America

Background: Congenital CMV infection (cCMV) is an important cause of sensorineural hearing loss (SNHL). Only limited data from newborn screening studies are available to describe newborn disease

and hearing outcomes.

Objective: To describe the newborn clinical findings and examine the association between newborn disease and hearing loss in a large cohort of infants identified on newborn screening.

Methods: In a prospective multicenter study, newborns at 7 U.S. hospitals were screened for cCMV. Clinical, laboratory and imaging data were collected from newborn medical records, examination at the initial follow up visit and imaging/laboratory studies completed after discharge from the hospital. Infants were considered to have symptomatic cCMV infection if they had any of the following symptoms in the newborn period: generalized petechial rash, purpuric rash, hepatomegaly, splenomegaly, jaundice with direct bilirubin ≥ 3 mg/dL, unexplained neurologic/CNS abnormalities, or chorioretinitis. Newborn disease was categorized as mild or moderate/severe based on the recent consensus guidelines (Lancet Infect Dis, In Press)

Results: Of the 449 infants with confirmed cCMV, 10% (44/449) had symptomatic infection. 41% of symptomatic infants (18/44) resided in the NICU. The most frequent finding was microcephaly in 38.6% (17/44). A generalized petechial rash, jaundice, hepatomegaly, and splenomegaly were observed in 27%, 21%, 25%, and 32%, respectively. Three infants had seizures and one had chorioretinitis. Symptomatic cCMV was categorized as mild in 43% (19/44) and moderate/severe in the remaining 57%. SNHL occurred in 21% of mild symptomatic infection compared with 48% of moderate/severe symptomatic infection ($p=0.06$).

Conclusions: In a large cohort of infants with cCMV identified by newborn screening, symptomatic infection occurred in 10% of infants, with nearly half of symptomatic infection classified as mild. Children with moderate/severe symptomatic cCMV had greater than 2-fold increased risk for SNHL.

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Date: 01/05/2017

Time: 10:55 - 12:55 hrs

B-INV-001

How, when, and why CMV acts like an RNA virus

Timothy Kowalik

University of Massachusetts Medical School, Microbiology and Physiological Systems, WORCESTER, United States of America

A major limitation of studies of infectious diseases is real-time access to sites of infection in patients. This challenge is exacerbated in congenital infections where fetal samples are generally unavailable during gestation. Population genetics uses the distribution and changes in allele frequencies to build statistically robust models to describe historical events that have shaped the sampled populations. Recent applications of this approach include recalibration of the timing and scale of ancient human migrations and the discovery of alleles under positive selection in humans. These tools have been refined for clinical viral infections, permitting analyses of evolutionary dynamics within individuals. By deep sequencing cytomegalovirus (CMV) populations from congenitally infected infants, we discovered that like RNA viruses, CMV exists as complex and evolving populations in vivo. Population genetics modeling of congenital CMV infection histories revealed the number and timing of bottlenecks representing maternal-fetal transmission and viral spread through fetal tissues. The bottleneck sizes suggest 10's to 100's of viral founders, which likely explain how CMV maintains diversity within and across individuals. This is in contrast to RNA viruses, which typically have founder sizes of 1-10 individuals. Given the low founder size, RNA viruses create intrahost diversity via high mutation rates. The modeling also produces inferences of where selection has acted on CMV populations. In sum, population level deep sequencing and modeling produce critical measures of infection history that do not require risky intrauterine sampling and are consistent with clinical features of congenital CMV infections. This approach is generalizable to the study of any viral infection and has application to the development of therapies and vaccines that limit disease.

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BOTH ORAL & POSTER PRESENTATION

B-OP-001

Role of cytoplasmic receptors NOD1 and NOD2 in CMV control

Ravit Boger, Arun Kapoor, Sujayita Roy, Rupkatha Mukhopadhyay

Johns Hopkins University School of Medicine, Pediatrics, BALTIMORE, United States of America

Episodes of CMV colitis in children with Inflammatory Bowel Disease and young infants with no apparent immunodeficiency prompted investigation of underlying genetic susceptibility to CMV. NOD2 is a susceptibility marker for Crohn's disease, and signals through the receptor interacting serine/threonine protein kinase (RIPK2), resulting in NF- κ B activation. Our studies revealed that NOD2 was induced by CMV at and after 12 h. NOD2 induction by CMV appeared to be a cellular defense mechanism as it resulted in virus suppression. The bacterial moiety muramyl dipeptide, which binds to and activates NOD2, inhibited CMV replication in a time- and concentration-dependent manner. CMV inhibition through NOD2 occurred via IFN- β dependent pathway, since in IFN- β knockdown cells NOD2 activation could not suppress CMV. Overexpression of a NOD2 mutant associated with severe Crohn's, failed to localize NF- κ B into the nucleus and to phosphorylate IRF3, and could not inhibit CMV. Survival of mouse CMV-infected NOD2 knockout mice was impaired compared to infected wild-type mice. Activation of the sister molecule, NOD1, with tri-DAP early after infection also suppressed CMV via IFN- β dependent pathway, requiring IKK α activation. NOD1 activation in mice inhibited mouse CMV replication and improved survival. A mutation in NOD1 that disrupts its interaction with RIPK2 resulted in failure of tri-DAP to inhibit CMV in vitro. Single nucleotide polymorphisms in NOD1 were significantly associated with CMV acquisition in a cohort of 400 CMV-seronegative women who participated in a CMV vaccine trial. Of 768 selected SNPs in 29 innate immune response genes, only 6 SNPs were significantly different between infected and non-infected women, 3 of which were in NOD1. Summarized, both NOD1 and NOD2 participate in CMV control via IFN- β induced pathway. Studies are ongoing to identify viral and cellular proteins that interact with NOD1 and/or NOD2 and which interferon stimulatory genes play a role in CMV suppression.

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BOTH ORAL & POSTER PRESENTATION

B-OP-002

The Human Cytomegalovirus tegument protein pp65 (pUL83) dampens interferon-type I production by inactivating the DNA sensor cGAS

Valentina Dell'Oste¹, Matteo Biolatti¹, Sara Pautasso¹, Francesca Gugliesi¹, Marco De Andrea¹, Jens Von Einem², Marisa Gariglio³, Santo Landolfo¹

¹Università degli Studi di Torino, TORINO, Italy

²University of Ulm, Institute of Virology, ULM, Germany

³University of Eastern Piedmont, Translational medicine, NOVARA, Italy

The innate immune response against Human Cytomegalovirus (HCMV) plays a pivotal role during primary infection. Indeed, HCMV infection of primary fibroblasts rapidly triggers a strong induction of interferons-type I (IFN-type I) accompanied by proinflammatory cytokine release. Here, we show that primary Human Foreskin Fibroblasts (HFFs) produce IFN-type I when infected with the wild type HCMV strain TB40/E (v65Rev). However, significantly higher IFN-type I levels were observed when HFFs were infected with HCMV unable to express UL83-encoded pp65 (v65Stop), suggesting that the tegument pp65 protein may downregulate IFN-type I production. To clarify the mechanisms pp65 relies on to inhibit IFN-type I production, we analyzed the activation of the cGMP--AMP synthase (cGAS)/STING/IRF3 axis in HFFs infected with v65Rev or with v65Stop. The results obtained reveal that pp65 selectively binds to cGAS and prevents its interaction with STING, thus inactivating the signaling pathway through the cGAS/STING/IRF3 axis. Notably, during the first six hours of HCMV infection, STING undergoes proteasome degradation regardless of the presence of pp65. Additionally, down-regulation of cGAS activity by pp65 blocked IRF3 dimerization and DNA binding activity, leading to the

inhibition of IFN-type I production. Collectively, our data provide mechanistic insight into the interplay between HCMV pp65 and cGAS leading to subsequent immune evasion by this prominent DNA virus.

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BOTH ORAL & POSTER PRESENTATION

B-OP-003

Human cytomegalovirus genome replication is inhibited in mature Langerhans-type dendritic cells.

Laura Hertel, Melissa Galinato, Olabisi Osunmakinde

Children's Hospital Oakland Research Institute, OAKLAND, United States of America

Langerhans-type dendritic cells (LC) are the only type of innate immune cells present in the outer layers of human oral epithelia. As such, they are likely to play important roles during CMV acquisition and spread, as well as in the mounting of adaptive immune responses. We previously reported that LC exposure to stimuli similar to those normally encountered in the oral cavity increases the proportion of infected cells, but dramatically reduces viral yields. This was not due to defects in progeny release, but to diminished progeny production rates. Here, we show that expression of the late and true-late proteins pp150 and pp28 at day 6-8 post-infection occurred in a 2-4-fold lower proportion of mature LC (mLC), as compared to immature LC (iLC). The number of cells containing actively replicating viral DNA as indicated by EdU incorporation was also similarly reduced, as was the percentage of mLC expressing the viral single-stranded DNA binding protein UL57. By contrast, no difference was detected in the percentage of cells expressing the early protein UL84, reportedly required for initiation of lytic DNA replication, or the viral polymerase processivity factor UL44. These data suggest that expression of some, but not all, of the viral replication complex components is impaired in mLC, leading to reduced viral genome replication rates, and that this may be due to the selective upregulation of specific restriction factors in mLC. While most cellular defense mechanisms known to date act by preventing virion entry and/or viral immediate-early gene expression, blocking viral replication is a novel and potentially advantageous means for innate immune cells to obtain a large pool of viral antigens for presentation to T and B cells, simultaneously preventing cell death and virus spread.

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BOTH ORAL & POSTER PRESENTATION

B-OP-004

CMV abrogates CD8 T-cell control by inhibiting apoptosis, not by inhibiting antigen presentation

M. Zeeshan Chaudhry¹, Rosaely Casalegno-Garduño¹, Stephan Halle², Iryna Dekhtiarenko¹, Julia Sonnemann¹, Ilija Brizic³, Stipan Jonjic³, Edward Mocarski⁴, Luka Cicin-Sain¹

¹Helmholtz Center for Infection studies, Vaccinology, BRAUNSCHWEIG, Germany

²Hannover Medical School, HANNOVER, Germany

³Faculty of Medicine, University of Rijeka, RIJEKA, Croatia

⁴Emory Vaccine Center, Emory University, ATLANTA, United States of America

Cytomegalovirus (CMV) induces CD8 T-cell responses of unparalleled strength, which decisively contribute to virus control. Cytomegalovirus encodes numerous immune evasion genes, for instance viral regulators of antigen presentation (vRAP) (e.g. m06, m152), which protect the virus from recognition by CD8 T-cells. We have shown that the murine cytomegalovirus (MCMV) lacking the gene inhibiting cell-extrinsic apoptosis (M36), shows severe growth defect *in vivo*. We hypothesized that M36 may protect MCMV infected cells from CD8 T-cell induced apoptosis.

We developed an *in vitro* co-culture system of MCMV-specific CD8 T cells and virus-infected fibroblasts or endothelial cells. The CD44⁺CD62L⁻ effector memory CD8 T cells isolated from latently infected mice failed to control the growth of wildtype MCMV or of the MCMV lacking vRAPs (Δ vRAP MCMV). However, the same CD8 T cells controlled the growth and spread of Δ M36-MCMV.

In vitro time-lapse imaging of co-culture systems showed that the Δ M36-MCMV infected cells are controlled by T cells via induction of apoptosis in infected cells, whereas no apoptosis occurred in MCMV^{WT} infection. Δ M36-MCMV growth was rescued by a pan-caspase inhibitor. Furthermore, CD8 T cells were unable to control Δ M36 MCMV growth in mouse embryo fibroblast (MEF) cells lacking caspase-8. A recombinant MCMV expressing dominant-negative FADD instead of M36 was controlled by CD8 T-cells, but only in the presence of IL2. This control was absent if perforin-deficient CD8 T-cells were used for co-culture experiments. Hence, our results suggest that CD8 T cells induce apoptosis in a caspase-8 dependent manner in the MCMV infected cells. T-cells induce apoptosis by death-receptor ligands and upon IL2 stimulation by the FADD-independent perforin/granzyme pathway. Taken together, the inhibition of caspase 8 dependent apoptosis, rather than of antigen presentation, is a key determinant of MCMV fitness in the presence of CD8 T cells.

250 - Session B.02: Virus and Host

Date: 01/05/2017

Time: 14:15 - 15:45 hrs

Invited presentation

B-INV-003

Cytomegalovirus (CMV)-encoded Fc γ Receptors: Novel Modulators at the Interface of Innate and Humoral Immunity

Hartmut Hengel, Philipp Kolb, Katja Hoffmann

Institute of Virology, Albert-Ludwigs-University, FREIBURG, Germany

The constant region of IgG, Fc γ , mediates vital antiviral functions including the engagement of host Fc γ R_s expressed by a variety of immune cells. Here we demonstrate HCMV to use sophisticated strategies of evasion from IgG-mediated control by expressing two viral Fc γ Receptors (vFc γ R_s) on the plasma membrane of infected cells, gp34/TRL11 and gp68/UL119-118 (Atalay et al., 2002). Using a recently developed reporter cell-based Fc γ R activation assay (Corrales-Aguilar et al., 2013) we found a robust inhibition of IgG-mediated triggering of activating human Fc γ R_s (CD16/Fc γ R_{III}, CD32A/Fc γ R_{II}, CD64/Fc γ R_I) by gp34 and gp68 (Corrales-Aguilar et al., 2014). Moreover, we provide evidence for a third vFc γ R, gp95/TRL12. Intriguingly, TRL12 represents one of the most polymorphic genes found among HCMV clinical isolates, but its ability to impair host Fc γ R activation appears nevertheless conserved. vFc γ R_s may explain the limited protective efficacy of HCMV hyperimmunoglobulin observed in various clinical settings. gp34 and gp68 form ternary complexes with immune IgG and antigen. According to the common 'antibody bipolar bridging' (ABB) hypothesis, their most obvious molecular mechanism of inhibition would be competitive Fc γ binding preventing host Fc γ R_s from recognizing the Fc γ domain. To address this important issue and to test whether both inhibitors act in a redundant way we compared Fc γ binding of host Fc γ R_s in the presence or absence of vFc γ R_s. We developed a test system using CD20 as a model antigen and anti-CD20 Rituximab to allow ABB complex formation upon transient expression of gp34 or gp68. Binding of soluble CD16 to ABB-complexes was readily detected by FACS and found unimpaired in the presence of gp34 but strongly reduced by gp68. Overall, our data suggest distinct modes of action for both vFc γ R_s and a division of labor between gp34 and gp68. The data support a novel concept of non-redundant but cooperative immune evasion mechanisms from IgG-Fc mediated effector responses.

250 - Session B.02: Virus and Host

Date: 01/05/2017

Time: 14:15 - 15:45 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-005

The contribution of CMV infection to immune senescence is set by the initial viral inoculum

Anke Redeker¹, Suzanne Welten¹, Esmé Van der Gracht¹, Ester Remmerswaal², René Van Lier², Ramon Arens¹

¹Leiden University Medical center, Immunohematology and Blood Transfusion, LEIDEN, Netherlands

²Academisch Medisch Centrum, AMSTERDAM, Netherlands

Age-associated immune alterations include impaired T cell responses and can as a consequence lead to increased disease susceptibility and decreased efficacy to vaccines. It is suggested that by promoting accumulation of memory CD8 T cells, persistent human cytomegalovirus (HCMV) infection is associated with an accelerated onset of immune senescence. However, evidence for this relationship remains elusive. Using mouse CMV (MCMV) as a model, we have investigated the influence of lifelong chronic CMV infection on antiviral immunity and addressed in particular the impact of the infectious dose. We show that the degree of CMV-specific memory CD8 T cell accumulation and the phenotypic T cell profile are directly influenced by the initial infectious dose, and data on HCMV-specific T cells suggest a similar connection. Moreover, following long-term infection with MCMV, the strength of the CD8 T cell response to superinfection with lymphocytic choriomeningitis virus (LCMV) inversely correlates with the height of the initial inoculum with MCMV. This coincides with a lower degree of activation of the LCMV-specific CD8 T cells that are induced when the initial MCMV inoculum increased, as was evidenced by decreased upregulation of CD27 and lower IL-2 production and consumption. Additionally, we show that long-term MCMV infection negatively affects the B cell response against LCMV in a dose-dependent manner, as evidenced by decreased IgM serum levels. Most importantly, we demonstrate that in latent MCMV infection the capacity to respond to superinfection with LCMV is impacted by the size of the initial inoculum. Taken together these results suggest that the contribution of CMV infection to immune senescence is set by the initial viral inoculum, and only substantial in case of exposure to a high initial dose. Thus, stratification based on the size and phenotype of the CMV-specific memory T cell pools is of interest to consider with respect to immune senescence.

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Date: 01/05/2017

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BOTH ORAL & POSTER PRESENTATION

B-OP-006

Simultaneous analysis by mass cytometry (CyTOF) of impact of human cytomegalovirus-encoded homologs of hIL-10 on multiple cellular signalling pathways

Selmir Avdic¹, Helen McGuire², Diana Shinko³, Lauren Stern¹, Caryn Van Vreden⁴, Brian McSharry¹, Megan Steain¹, Kellie Charles³, Barbara Fazekas de St Groth², Allison Abendroth¹, Barry Slobedman¹

¹Discipline of Infectious Diseases and Immunology, the University of Sydney, CAMPERDOWN, Australia

²Centenary Institute of Cancer Medicine and Cell Biology, CAMPERDOWN, Australia

³Discipline of Pharmacology, the University of Sydney, CAMPERDOWN, Australia

⁴Centenary Institute Medical Research Foundation, CAMPERDOWN, Australia

The human cytomegalovirus (HCMV) gene UL111A encodes two homologs of human immunosuppressive cytokine interleukin 10 (hIL-10), termed cmvIL-10 and LAcmvIL-10. We and others have previously reported a range of immunomodulatory functions exhibited by cmvIL-10, while biological functions of LAcmvIL-10 are more restricted. Due to technical limitations, most of previous studies were performed either on a single cell subset or looking at a single cellular target within a mixed population of cells. Development of mass cytometry (CyTOF) allowed us to perform multiplexed analysis of cell subset-specific signalling pathway activation 'signatures' induced by cmvIL-10 and LAcmvIL-10 in peripheral blood mononuclear cells (PBMCs). Our 36-antibody CyTOF panel was designed to identify most major cell subsets present in PBMCs and it also included antibodies detecting phosphorylation (i.e. activation) of eight signalling pathways in each PBMC subset.

Two major questions asked in our study were:

Which signalling pathways are phosphorylated in different PBMC subsets by cmvIL-10 and LAcmvIL-10?

Can cmvIL-10 and LAcmvIL-10 modulate activation of a number of signalling pathways triggered by

numerous host cytokines and if so, which PBMC subsets are targeted?

In addition to previously reported Jak1/Stat3 and PI3K/Akt signalling pathway phosphorylation by cmvIL-10 in CD14⁺ monocytes, our study identified Stat3 phosphorylation by cmvIL-10 in a number of other PBMC subsets. Furthermore, we have also identified Stat5 phosphorylation by cmvIL-10 in some, but not all PBMC subsets, which was subsequently suppressed by prolonged exposure to cmvIL-10. LAcmvIL-10 had more restricted capacity to phosphorylate signalling pathways tested in our study. Instead, LAcmvIL-10 appeared to prevent Stat3 phosphorylation by hIL-6 specifically in CD14⁺ monocytes and inhibit global Stat1 phosphorylation by IFN-gamma.

In summary, this study reports for the first time phosphorylation of Stat5 associated with cmvIL-10 treatment and identifies signalling pathways that may be signalling atypically in presence of LAcmvIL-10.

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Date: 01/05/2017

Time: 14:15 - 15:45 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-007

Cytomegalovirus-encoded Death Suppressors Cooperate to Prevent Proinflammatory Consequences of Combined Apoptosis and Necroptosis.

Edward Mocarski¹, Lisa Daley-Bauer¹, Linda Roback¹, Lynsey Crosby¹, Louise McCormick¹, Yanjun Feng¹, William Kaiser²

¹Emory University, Department of Microbiology and Immunology, ATLANTA, United States of America

²University of Texas Health Science Center, Department of Microbiology and Immunology, SAN ANTONIO, United States of America

Caspase-8 drives extrinsic apoptosis and prevents receptor-interacting protein kinase (RIPK)3-dependent necroptosis. These cell death pathways have antiviral as well as inflammatory consequences that remain poorly understood. Cytomegaloviruses encode suppressors of apoptosis and necroptosis during infection to sustain infected cell viability. The murine CMV inhibitor of caspase-8 activity (vICA, encoded by M36) is conserved in human CMV, and the viral inhibitor of RIP activation (vIRA, encoded by M45) is conserved in the alphaherpesvirus, HSV. Here, we have investigated the consequences of vICA and vIRA together in murine CMV in cultured cells as well as in the naturally infected mouse host. vICA-deficient virus and vIRA-deficient viruses showed expected susceptibility to apoptosis and necroptosis, respectively. In contrast, double mutant virus activates caspase-8 to trigger apoptosis that gives rise to secondary necroptosis. Infection with double mutant Δ M36/M45mutRHIM MCMV reveals a signaling pattern where caspase-8 activates caspase-3 to drive apoptosis with subsequent RIPK3-dependent activation of mixed lineage kinase domain-like (MLKL) leading to necroptosis. This combined death signaling is highly inflammatory, greater than either apoptosis induced by vICA-deficient virus or necroptosis induced by vIRA-deficient MCMV. Double mutant virus drives significant increases in IL-6 production in infected macrophage cultures and elicits faster anti-viral responses in the host compared to wild type or single mutant virus infection. Collaboratively, vICA/M36 and vIRA/M45 target caspase-8 and RIPK3 signaling pathways together in order to suppress proinflammatory cell death. This study clarifies the impact of caspase-8 activation on the unleashing of necroptosis and also identifies a novel anti-viral mechanism of caspase-8 activation in concert with RIP3 signaling in driving proinflammatory cell death.

250 - Session B.02: Virus and Host

Date: 01/05/2017

Time: 14:15 - 15:45 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-008

Mast cells as rapid innate sensors of cytomegalovirus by TLR3/TRIF signaling-dependent and -independent mechanisms

Niels Lemmermann, Ann-Kathrin Hartmann, Michael Stassen, Matthias J. Reddehase

The traditional perception of mast cells (MC) was dominated for decades by their notorious property to initiate IgE-dependent allergic reactions. However, it is increasingly being appreciated that MC are an integral part of innate immunity, based mainly on their aptitude to rapidly initiate acute inflammatory reactions in the absence of IgE antibodies. MC are located at critical sites of endothelial and epithelial surfaces, and are equipped with an arsenal of pattern recognition receptors. They store a wide range of potent pro-inflammatory and antimicrobial mediators in secretory granules that can be instantly released upon activation. Therefore, MC are able to cross-talk with cells from both the innate and adaptive arms. Recently, we provided evidence for a cross-talk axis between MC and adaptive immune defense in the lungs of murine cytomegalovirus (mCMV)-infected mice. Infection of MCs results in MC degranulation and release of the chemokine CCL5/RANTES. The chemokine release enhances the recruitment of protective CD8 T cells to extravascular sites of virus replication, specifically the lung interstitium and alveolar epithelium, thereby enhancing the control infection.

Using TRIF^{-/-} and TLR3^{-/-} mice, we defined two temporally and mechanistically distinct waves of MC activation by mCMV infection. An early (4h p.i.) activation of MCs mediated by TLR3/TRIF signaling and a delayed (24h p.i.) TLR3/TRIF-independent second round of activation triggered by direct MC infection. For the latter we have hints that mCMV gene products that modulate the cytoplasmic Ca²⁺ levels trigger the MC activation.

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Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-001

Characteristics of newborns born with congenital cytomegalovirus infection after multiple-birth pregnancy

Efraim Bilavsky¹, Einat Shmueli¹, Eran Hadar², Joseph Pardo², Nehama Linder¹, Joseph Attias¹, Jacob Amir¹

¹Schneider Children's Medical Center of Israel, PETAH TIQVA, Israel

²Helen Schneider Hospital for Women Rabin Medical Center, Beilinson Hospital, Department of Gynecology and Obstetrics, PETAH TIQVA, Israel

Background: Cytomegalovirus (CMV) infection is the most common cause of congenital infection, the most common non-genetic cause of childhood sensorineural hearing loss and an important cause of neurodevelopmental delay. While data on vertical transmission and neonatal outcome after singleton pregnancy is well established, only scarce reports assessed the issue of congenital CMV (cCMV) in multiple birth pregnancies. Moreover, there are no studies which compared the outcome after birth as well as long term follow up of children with cCMV born after singleton vs. multiple pregnancy.

Methods: Data of all infants with cCMV infection between 2005 and 2015 were reviewed. Outcome after birth of symptomatic vs. asymptomatic disease was compared between infants born after multiple (study group) and singleton (control group) pregnancy in a 1:2 ratio.

Results: Of 508 infants diagnosed with cCMV in our clinic, there were 25 (4.9%) born after multiple pregnancy (study group). Children from the study and control group did not differ in term of specific prenatal CMV investigation including amniocentesis and brain Magnetic Resonance Imaging Studies. There was a higher rate of symptomatic cCMV infection in the study group than in the control group (48% vs. 14%, p<0.001). Hearing impairment at birth was also more frequent in the study group (32% vs. 8%, p=0.016). Rate of other CNS manifestations and rate of non CNS manifestations were not statistically different between the groups at birth. On a long-term follow up children in the study group had higher rates of neurological sequelae (hearing impairment or neurodevelopmental delay) compared to children in the control group (20% vs. 4%, p=0.016).

Conclusions: Infants with cCMV born after multiple birth pregnancies have higher risk for symptomatic disease at birth and worse long-term neurological outcome than those born after singleton pregnancy. This important group of children deserves a close prenatal and postnatal care.

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Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-004

Cytomegalovirus reactivation in pediatric acute leukemia after stem cell transplantation has an effect on relapse and survival in AML

Sebastian Voigt¹, Laura Sparkuhl², Jörn-Sven Kühl²

¹Robert Koch Institute/Charité, Infectious Diseases/Ped Oncology/Hematology, BERLIN, Germany

²Charité - Universitätsmedizin Berlin, BERLIN, Germany

Several studies have indicated better survival after stem cell transplantation (SCT) for acute leukemias, especially acute myeloid leukemia (AML), in case of cytomegalovirus (CMV) reactivation. To investigate if CMV reactivation after SCT for childhood ALL or AML influences relapse rates, we analyzed data from 177 pediatric allogeneic stem cell transplant recipients from our institution who received myeloablative conditioning. Transplant indications included AML, T-ALL and B-precursor ALL. CMV reactivation was correlated with relapse as well as other factors. 42 patients were transplanted for AML (24 %), 22 for T-ALL (12 %), and 113 for B-precursor ALL (64 %). Mortality and relapse rates (27-37% and 18-26%, respectively), CMV reactivation rates (21-36%) as well as numbers of negative CMV serology status (19-32%) of donor and recipient were comparable between different acute leukemias. When patients were analyzed altogether, CMV reactivation had no effect on relapse rates or mortality. However, a tendency towards fewer relapses after CMV reactivation was observed in AML patients (no relapse (0 %) with CMV reactivation vs. 11 relapse cases (33 %) without CMV reactivation; $p=0,083$). In those 128 leukemia patients capable of reactivating CMV (i.e., donor or recipient CMV seropositive prior to SCT), CMV reactivation had a protective effect on relapse rates in AML (no relapse (0 %) with CMV reactivation vs. 11 relapse cases (44 %) without CMV reactivation; $p=0,017$). A similar tendency could be seen in T-ALL whereas no effect in patients with B-precursor ALL was documented. Latently CMV infected AML patients without documented CMV reactivation after SCT have a significant worse prognosis compared with all other AML patients. This is also likely to be the case in patients with T-ALL. The protective effect of CMV reactivation in AML and possibly T-ALL does not appear to be GVH-related. CMV reactivation after SCT for B-precursor ALL lacks significance.

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Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-005

Urinary Cytomegalovirus Shedding in the United States: the National Health and Nutrition Examination Surveys, 1999-2004

Chengbin Wang, Chengbin Wang, Minal Amin, Tatiana Lanzieri, Stephanie Bialek, Sheila Dollard
Centers for Disease Control and Prevention, ATLANTA, United States of America

Background Shedding of cytomegalovirus (CMV) has been well documented in specific sub-populations, but to date there have been no prevalence estimates reported for a US population sample.

Method Urine specimens from CMV IgG+ participants aged 6-49 years of three racial/ethnic groups (non-Hispanic white, non-Hispanic black, and Hispanic [Mexican-American]) from the National Health and Nutrition Examination Surveys (NHANES) 1999-2004, were tested for the presence of CMV DNA with quantitative PCR.

Results Among 6828 CMV IgG+ subjects tested, 537 had CMV DNA detected in the urine for a shedding prevalence of 6.8% among CMV IgG+ subjects and 2.7% among all NHANES subjects (n=14190) under the assumption that all untested urine specimens from CMV IgG- subjects would be negative. Prevalence of urinary shedding in those aged 6-11, 12-19, 20-29, 30-39, and 40-49 years was

statistically different among CMV IgG+ subjects (26.8%, 6.5%, 3.9%, 4.5%, and 2.3%, respectively, $P < 0.001$) and among all subjects (8.2%, 2.3%, 1.7%, 2.2%, and 1.2%, respectively, $P < 0.001$). In addition, children aged 6-11 years had higher urinary viral loads than other age groups (arithmetic mean: 27282, 7199, 2613, 5121, and 258 copies/mL in those aged 6-11, 12-19, 20-29, 30-39, and 40-49 years, respectively; $P < 0.001$), which persisted in all three racial/ethnic groups.

Conclusions Urinary CMV shedding varied dramatically by age with the highest prevalence and viral loads among children aged 6-11 years, which likely reflects higher rates of primary CMV infection. The findings demonstrate that children continue to shed CMV at relatively high rates beyond 5 years of age and thus may be an important source for CMV transmission.

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Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-009

A CMV-like virus is implicated in head and neck paraganglioma, a highly vascular tumor of the autonomic nervous system

Mattia Russel Pantalone¹, Simone Vespa², Fabio Verginelli³, Silvia Perconti¹, Diana Liberata Esposito¹, Rosalba Florio³, Alessandro Cama³, Lorenzo Tramontana², Artenca Sheu¹, Michele Serluca¹, Marco Marchisio¹, Paola Lanuti¹, Afsar Rahbar⁴, Sampath Chandra Prasad⁵, Rosa Visone¹, Carlo T. Paties⁶, Mario Sanna⁵, Cecilia Söderberg-Nauclér⁴, Lavinia Vittoria Lotti², Renato Mariani-Costantini¹

¹CeSI-MeT, 'G. d'Annunzio' University, Medical, Oral and Biotechnological Sciences, CHIETI, Italy

²„ Sapienza,, University of Rome, Experimental Medicine, ROME, Italy

³G. d'Annunzio' University, Pharmacy, CHIETI, Italy

⁴Karolinska Institutet, CMM, Karolinska University Hospital, Medicine, STOCKHOLM, Sweden

⁵Gruppo Otologico Piacenza-Roma, Otology and Skull Base Unit, ROME, Italy

⁶„ Guglielmo da Saliceto,, Hospital, Anatomical Pathology, PIACENZA, Italy

Paragangliomas (PGLs) are neurovascular autonomic nervous system tumors, still incurable when radical surgery is not possible. They are frequently associated with susceptibility mutations in one of several nuclear genes, notably those encoding the mitochondrial SDH complex components. However, the etiology of PGLs remains unclear, as genetically-modeled mice with *Sdh* mutations do not develop cancer (Piruat and Millán-Uclés, 2014). Studying a large set (n=68) of freshly-collected head and neck PGLs (HNPGs) by electron microscopy we serendipitously found herpesvirus-like particles in all tumors. These were similar to particles attributed to cytomegalovirus (CMV) reported back in 1971 by Heine et al. in a retroperitoneal PGL. Although our attempts to reveal the viral code via PCR using human CMV-specific primers remain incompletely resolved, the presence of a CMV-like virus in our HNPG series, as detected by ultrastructural analysis, was supported by: 1. presence of several CMV reactive proteins (IE, gB, pp65, vMIA) and interacting cell proteins (PDGFRA, viperin); 2. detection of a short conserved stretch corresponding to the CMV gB sequence via PCR; 3. in situ hybridization using a large CMV-specific probe. Moreover, patient-derived xenografts presented the same viral particles and proteins found in the HNPGs of origin and CMV gB sequences were retrievable from the blood of xenografted mice. Ganciclovir and Imatinib arrested the growth of HNPG cell cultures, inducing cell death with decrease in the load of viral and interacting cellular proteins, and efficiently prevented HNPG xenograft formation. Coherently with emerging evidence concerning other neural cancers (Söderberg-Nauclér and Johnsen, 2015), our data suggest that a CMV-like virus plays a role in the pathogenesis of HNPG, possibly favoured by germline mutations predisposing to oncogenic infection.

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Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
A-PP-014

Congenital CMV infection in Finland - a prospective cohort study

Laura Puhakka¹, Maija Lappalainen¹, Riina Niemensivu¹, Päivi Lindahl¹, Tuula Lönnqvist¹, Raija Seuri¹, Irmeli Nupponen¹, Tea Nieminen¹, Sunil Pati², Suresh Boppana², Harri Saxen¹

¹Helsinki University Hospital, Pediatrics, HELSINKI, Finland

²University of Alabama at Birmingham, Pediatric Infectious Diseases, BIRMINGHAM, AL, United States of America

Background: Congenital cytomegalovirus infection (cCMV) may lead to severe morbidity including hearing loss, visual impairment and neurological abnormalities. Majority of the infected infants are asymptomatic. For evaluating the benefits of universal newborn screening, it is essential to understand the disease burden of cCMV.

Objective: To determine the prevalence of cCMV and study the outcome of infected infants at 18 months of age.

Methods: 19,868 newborns were screened by saliva CMV real-time PCR between September 2012 and January 2015. The CMV-positive infants and matched controls were followed up to determine neurological, audiological and ophthalmological outcome. Griffiths development scales, otoacoustic emission and sound field audiometry, and ophthalmologic examination were performed.

Results: Out of 19,868 infants screened, 40 were confirmed to have cCMV for a prevalence of 0.2% (95% CI 0.14 – 0.26 %). The neurological follow-up was completed by 37 patients and 51 controls, audiological follow-up by 35 patients and 46 controls, and ophthalmological follow-up by 35 patients and 47 controls. The outcome did not differ between patients and controls. The mean Griffiths general quotient for patients was 101.0 and controls 101.6 (p=0.752). No difference in any subscale was observed (locomotion, personal-social, hearing and language, eye and hand, performance) (p=0.25 – 0.916). Otoacoustic emission failed in 4/54 positive and 6/80 negative ears (p=1.000) and no difference in the mean thresholds in sound field audiometry was observed (34.31 dB for patients and 32.73 for controls) (p=0.133). No CMV related ophthalmologic findings were observed.

Conclusions

The burden of cCMV infection in Finland was lower than expected. The prevalence (0.2%) was lower and the outcome at 18 months of age did not differ significantly from controls. Universal screening for cCMV seems therefore unwarranted in Finland. The subsequent follow-up will determine the occurrence of late onset hearing losses at 3 and 6 years.

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Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
A-PP-016

Outcome of HCMV Non-Primary Infection in Pregnancy: Incoming Data from China and Implication on the View in the Scientific Community

Gerhard Jahn¹, Ji Yang², Ye Lijing², Jin Xu²

¹University Hospital of Tübingen, Institute for Medical Virology and Epidemiology of Viral Disease, TÜBINGEN, Germany

²Childrens' Hospital of Fudan University, Childrens' Hospital of Fudan University, SHANGHAI, China

Symptomatic HCMV congenital infections in newborns, born by mothers with preexisting antibodies are published from Alabama and Sao Paolo. The rate of bad outcome was about the same like in primary infection. Secondary infection is highly dependent on lifestyle such as close contact to children < 3 years and the number of sexual partners during pregnancy. These exposures can lead to acquire a new HCMV gp-type. In recent studies in HCMV seropositive mothers in China, done in two hospitals of Shanghai, we found that 17 out of 1780 investigated DBS were positive (0,9%). In 10 out of 17 PCR positive newborns follow ups could be done. None of the positive newborns had clinical symptoms of HCMV-infection. All newborns were screened at birth between Nov. 2011 to Dec. 2012 in the Obstetrics and Gynaecology Hospital of Fudan University and the Community Health Center of Pujiang Town. The

rate of newborn infection in CHC of Pujiang Town was 1,6% in contrast to only 0,2% of the newborns seen at the Hospital of Fudan University. The population of Pujiang was characterized by migrants with lower income and education levels in contrast to the population seen at Fudan University, which were characterized by higher incomes and higher education. Our findings are in agreement with recent published data from Turkey and from other data in China (Shandong Province), obtained by Sheila Dollard (CDC, Atlanta) and Chinese colleagues. These incoming data from China and Turkey (countries with high prevalence), are in clear contrast to published data from Alabama and Sao Paolo. The reasons for this conflicting data are most likely conditionally by lifestyle. This fact can explain the differences in the prevalence of HCMV secondary infection (close contact to children < 3 years and number of sexual partners during pregnancy) in the investigated population groups.

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Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-020

Degree and Audiometric Patterns of Cytomegalovirus-Related Sensorineural Hearing Loss in Children: Findings from the CHIMES Study

Karen Fowler¹, Shannon Ross¹, Amina Ahmed², April Palmer³, Marian Michaels⁴, David Bernstein⁵, Pablo Sanchez⁶, Kristina Feja⁷, Audra Stewart⁸, Suresh Boppana¹

¹University of Alabama at Birmingham, Pediatrics, BIRMINGHAM, United States of America

²Carolinas Medical Center, Pediatrics, CHARLOTTE, United States of America

³University of Mississippi Medical Center, Pediatrics, JACKSON, United States of America

⁴University of Pittsburgh & Children's Hospital of Pittsburgh, Pediatrics, PITTSBURGH, United States of America

⁵Cincinnati Children's Hospital Medical Center & University of Cincinnati, Pediatrics, CINCINNATI, United States of America

⁶Ohio State University & Nationwide Children's Hospital, Pediatrics, COLUMBUS, United States of America

⁷Saint Peter's University Hospital, Pediatrics, NEW BRUNSWICK, United States of America

⁸University of Texas Southwestern Medical Center, Pediatrics, DALLAS, United States of America

Background: Congenital CMV infection (cCMV) is an important cause of sensorineural hearing loss (SNHL). CMV-related SNHL may either be present at birth or occur later.

Objective: To describe the degree and audiometric patterns of CMV-related SNHL among infected infants.

Methods: Infants born at seven US medical centers participating in the NIDCD CHIMES study were screened for cCMV infection while in the newborn nursery. Infants who tested positive for CMV received follow-up diagnostic audiologic evaluations thru 48 months of life to identify or confirm hearing loss.

Results: SNHL in the neonatal period occurred in 35/449 (7.8%, 95% CI, 5.5 – 10.7%) of the cCMV infants with 45% having bilateral hearing loss. Among infants with SNHL who did not receive antiviral therapy (n=25), progressive or worsening hearing loss occurred in 60% (median=18 mo; range, 7 – 48 mo) and fluctuating hearing loss occurred in 12%. Similarly in infants treated with antivirals (n=10), progressive and fluctuating hearing loss occurred in 50% (median=30 mo; range, 7 - 48 mo) and 20%, respectively. An additional 15 children had late onset hearing loss, so the overall SNHL in cCMV children at 4 years of age was 11.1% (95% CI, 8.4 – 14.4%). Severe to profound hearing loss (> 70 dB) in at least one ear occurred in 68% of children with CMV-related SNHL. No standard audiometric configuration or pattern was observed. Over 80% of children with hearing loss had asymmetric loss where the degree and audiometric configuration was different in each ear.

Conclusion: CMV-related SNHL does not have a standard audiometric configuration and is mostly asymmetric. The severity of loss in at least one ear ranges from severe to profound hearing loss for the majority of children with CMV-related SNHL. CMV-related SNHL may continue to worsen during early childhood even in children who received antiviral therapy.

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Date: 01/05/2017

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POSTER PRESENTATION

A-PP-023

Human Cytomegalovirus can Promote Human Colorectal Cancer through reduced EphB2 Expression

Cecilia Söderberg-Naucler, Klas Straat, Mensur Dzabic, Chato Taher, Natalia Landázuri Sáenz, Piotr Religa, Ersen Kavak, Anna Martling, Jan Björk, Koon Chu Yaiw, Sandberg Rickard
Karolinska Institute, Dept of Medicine, STOCKHOLM, Sweden

Human cytomegalovirus (HCMV) has for a long time been suspected to play a role in the progression of human cancer. HCMV nucleic acids and/or proteins have been identified in various human cancers including colon cancer. Although growing evidence implies that HCMV is connected with human cancers and that HCMV proteins such as IE and US28 under certain circumstances induce oncogenic transformation, evidence are lacking that HCMV is oncogenic. Instead, HCMV is believed to be oncomodulatory, capable of modifying cellular processes leading to tumor initiation and/or tumor progression. Previous studies show that expression of the tumor suppressor erythropoietin-producing hepatocellular (Eph)-B2 receptor is low or absent during tumor development in human colon carcinoma (CCR). We and others have detected a high prevalence of HCMV protein expression in CCR, and in brain metastases of CCR. High grade of HCMV protein expression correlated with poor prognosis. We hypothesized that HCMV is responsible for the observed EphB2-loss in CCR, thereby promoting invasiveness and metastatic disease. We found a significant negative correlation between EphB2- and HCMV-protein levels in tissue specimens obtained CCR patients. Analysis of RNA deep sequencing data from 44 human colorectal tumors demonstrated reads aligning to the HCMV genome in 27% of these tumors. HCMV-positive tumors had fewer reads mapping back to the human EphB2 transcript as compared to the HCMV-negative tumors. In vitro analyses demonstrated that HCMV-infection significantly reduced EphB2 mRNA and corresponding protein levels in colon cancer cells by 80-90% and that infected cells exhibited a more invasive cell phenotype. Furthermore, MCMV-infection increased the metastatic capacity of mouse colon cancer cells in a metastatic mouse model, which was reversible by overexpression of EpHB2. These observations imply a potentially pivotal role of HCMV to control EpHB2 expression in invasive colon cancer. These observations may open for new treatment options for selected CCR patients.

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Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-024

Congenital CMV survey in general population in France

Sophie Alain¹, Elodie Loum-Ribot², Tiffany Lamblot³, Sebastien Hantz¹, Marianne Leruez-Ville⁴, French data base group Congenital CMV⁵

¹National Reference Center for Cytomegaloviruses, CHU Limoges, Virology, LIMOGES, France

²National Reference center for Cytomegalovirus, Virology, LIMOGES, France

³CNR CMV associated Laboratory, CHU Necker -Enfants malades, Virology, PARIS, France

⁴CHU Necker-enfants Malades, Virology, PARIS, France

⁵Study group, , France

In the absence of screening, estimating the burden of congenital cytomegalovirus infection in France and the heterogeneity of practices remains difficult. As a reference center we put in place a systematic declaration of congenital CMV cases since 2006. To improve the case declaration, an on-line version allowing pregnancy and infected children follow-up and treatment survey was developed with agreement of the French ethical committees in 2015. A case is a congenital infection diagnosed by amniotic fluid PCR or culture, or viruria at birth, or a retrospective diagnosis from Guthrie card. Cases are classified on the

basis of symptoms detected either by sonographic examination or clinically. Severe: neurologic abnormalities, mild: extra-neurologic symptoms or unilateral hearing loss, Asymptomatic: absence of sonographic or clinical symptoms. Updated results: We collected 817 cases between 2006 and 2015 (87 (+/-24) cases per year), from 28 prenatal diagnosis centers of 23 metropolitan and several overseas regions covering quite all the French territory. Interestingly, the severe (23%) and moderate cases (18%) remained stable, while not documented cases decreased from 43% to 22% with an increase of asymptomatic cases (18% to 23%). 116 cases were incorporated in the data base for 2015: 27(23%) asymptomatic, 64 (55%) symptomatic, 25 (22%) not documented. The symptomatic cases were 2 neonatal death (3%), 3 in utero death (5%), 23 hearing loss (35%), 2 neurological abnormality (3%), 8 mental disabilities (12%) and 18 medical abortions (28%). Amongst 12 newborns for which information is available only 2 received valganciclovir, while, as recently recommended, 46 could have been treated and some medical abortions could also have been avoided. We now collect data on other newborns treatment. Identifying the burden of symptomatic cases and follow-up of treated children through the data base could help to measure the benefits and consequences of valganciclovir newborns treatment in current practice.

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Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-002

Cytomegalovirus Seroprevalence among Children 1-5 Years of Age in the United States

Tatiana Lanzieri, Deanna Kruszon-Moran, Susanna Schmink, Sheila Dollard

Centers for Disease Control and Prevention, ATLANTA, United States of America

Aim: To assess the associations between sociodemographic factors and breastfeeding history with cytomegalovirus (CMV) seroprevalence among children 1-5 years of age in the United States.

Methods: We analyzed population-based data from the National Health and Nutrition Examination Survey, 2011-2012, using logistic regression to calculate adjusted odds ratios (aOR) and 95% confidence intervals (CI).

Results: Nationally, CMV IgG seroprevalence among children 1-5 years of age was 20.7% (95% confidence interval (CI), 14.4-28.2%). CMV seroprevalence was significantly higher with increasing age: 12.3% in 1 year-olds, 19.9% in 3 year-olds and 31.1% in 5 year-olds. CMV seroprevalence was 10.6% among non-Hispanic white children and significantly higher in every other racial/ethnic group: 24.5% among non-Hispanic black children, 31.0% among Hispanic children and 59.2% among non-Hispanic Asian children. CMV seroprevalence was significantly higher among children living below the poverty index compared to those at or above (aOR=2.3; 95%CI=1.4-4.0), and among children living with 1 or more vs. no other children \leq 5 years of age in the household (aOR=2.0; 95%CI=1.2-3.5). CMV seroprevalence was higher among children who were breastfed up to 6 months (aOR=1.4; 95%CI=0.9-2.1) or >6 months (aOR=3.1; 95%CI=1.3-7.5) compared to those who were not breastfed.

Conclusions: Among children 1-5 years of age, the following characteristics were independently associated with CMV seroprevalence: race/Hispanic origin, poverty, more young children in the household, and history of breastfeeding for >6 months.

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Date: 01/05/2017

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POSTER PRESENTATION

A-PP-003

Cytomegalovirus (CMV) shedding in seropositive pregnant women from a high seroprevalence population (BRACHS Study)

Marisa M Mussi-Pinhata¹, Nayara G Barbosa¹, Aparecida Y Yamamoto¹, Geraldo Duarte¹, Karen

Fowler², Suresh Boppana², William J Britt²

¹Ribeirão Preto Medical School, University of São Paulo, Pediatrics, RIBEIRÃO PRETO, Brazil

²University of Alabama, Pediatrics, BIRMINGHAM, United States of America

Background: Most congenital CMV infections occur in infants born to women with preexisting CMV seroimmunity (non-primary infection) in highly seropositive populations. Virologic and immunologic correlates of intrauterine transmission in non-primary infection are not known but essential for developing effective prevention strategies. CMV shedding patterns in pregnant women with non-primary infections have not been characterized. **Objective:** To determine the correlates of CMV shedding in a cohort of seropositive pregnant women. **Methods:** In a prospective study, 120 CMV seropositive pregnant women were monitored and specimens (saliva, urine, vaginal swabs, blood) collected in 1st, 2nd, and 3rd trimesters and one month postpartum. Specimens were tested by a qualitative PCR for CMV and confirmed by a quantitative CMV-PCR. Simple and multiple log-binomial regression models were used to analyze the contribution of maternal age, education, marital status, parity, contact with young children, the number of persons living in the household, sexual activity, and condom use on viral shedding. **Results:** Overall, 2,512 samples were tested from 120 subjects. CMV shedding was detected at least once in 35%(42) of these women. Maternal age, education, parity and marital status were not associated with viral shedding. Mothers living with or providing daily care to young children (3-6 years) were twice as likely to shed CMV than those not exposed to children (58% vs. 26%; aRR=2.21; 95%CI=1.37-3.56). Living in crowded households (≥2 people per room) was also associated with viral shedding (64% vs. 31%; aRR=1.99; 95%CI=1.26-3.13). However, sexual activity as indicated by the number of sexual partners per year or condom use was not associated with viral shedding. **Conclusions:** CMV shedding is frequent in seropositive pregnant women. The association between virus shedding and caring for young children as well as crowded living conditions suggests that increased exposures are important factors leading to CMV reinfections and intrauterine transmission in seropositive women.

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Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-007

Risks factors for congenital CMV following primary and non-primary maternal infection: a prospective neonatal screening study using PCR in saliva

Marianne Leruez-Ville¹, Jean-Francois Magny¹, Sophie Couderc², Christine Pichon¹, Marine Parodi¹, Laurence Bussières¹, Tiffany Guillemot¹, Idir Ghout³, Yves Ville¹

¹Hopital Necker-Enfants-malades, Virology, PARIS, France

²Hopital de Poissy, Maternity, POISSY, France

³Hospital Ambroise Paré, Statistitical Department, BOULOGNE, France

Background: There are gaps in the knowledge of cCMV epidemiology according to the type of maternal infection. The development of large-scale neonatal screening tools is an unmet challenge. This has consequences on the design of diagnostic and preventive strategies.

Methods and findings: 11,715 neonates were screened by CMV PCR in saliva during 2 consecutive years in France. Prevalence, type of maternal infection, socio-demographic, obstetrical and serological data were analyzed. Positive predictive value of CMV PCR in saliva was 59% (95% CI: 47.5, 69.1), false positive results were associated with lower viral loads ($p < 0.001$). Maternal seroprevalence was 61% (95% CI: 58, 62), birth prevalence was 0.37% (95% CI: 0.273, 0.504), 52% and 47.7% of cases were from primary and secondary infections respectively. The proportion of infected neonates with hearing loss was similar following maternal primary and secondary infection (8.6% v 9.5%, $p > 0.99$). The risk to deliver an infected baby after primary infection was increased in younger (OD=7.9 [2.6, 23.8]), multiparous (OD=4.1 [1.7, 10.0]) women born in high resources countries (OD=5.2 [1.5, 18.2]) and from higher income groups ($p = 0.019$). The only 2 risk factors to deliver an infected baby after secondary infection were to be young (OD=4.6 [1.5, 14.6]) and unemployed (OD=5.8 [2.2, 15.9]). The risk to deliver an infected baby was 4-fold higher in women seronegative before their pregnancy ($p = 0.021$).

Conclusions: A positive CMV PCR in newborns' saliva should always be confirmed in a repeat-sample.

Socio-demographic characteristics of women giving birth to an infected baby after primary and secondary infection are different. Seronegative, parous women represent the highest risk population for cCMV in countries with low to intermediate seroprevalence in pregnant women. Urgent action is needed to stop the epidemics of cCMV in general and particularly in this population that can be easily identified by serology and targeted by prevention messages.

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Date: 01/05/2017

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POSTER PRESENTATION

A-PP-008

Factors influencing the outcome of infants with congenital CMV Infection

Dimitra Dimopoulou¹, Kassiani Kekkou², Anastasia Antoniadou², Lampros Mariolis², Konstantinos Douros², Maria Tsilika², Efthymia Alexopoulou², Dimitra Kavatha², Vassiliki Papaevangelou²

¹University Hospital of Athens, ATTIKO, ATHENS, Greece

²ATTIKON UNIVERSITY HOSPITAL, ATHENS, Greece

Background: Cytomegalovirus (CMV) is the leading cause of congenital viral infection in the developed world, resulting in long-term disability, such as sensorineural hearing loss and cognitive deficit. The objective of this study is to evaluate potential factors associated with adverse outcome in congenital CMV infection (cCMV).

Patients and Methods: A total of 62 infants born to women infected with CMV during pregnancy, followed in our department, were retrospectively analyzed. Most women (57/62) had primary CMV infection and 48 received CMV-IG (1-9 doses). We examined whether timing of maternal infection (pregnancy trimester), sonographic findings, treatment of mother and offspring as predictors of outcome (cCMV infection, abnormal head US and hearing loss among cCMV infants).

Results: Gestational age at birth, timing of maternal infection and maternal treatment were not significant predictors of outcome ($p=0.65$, $p=0.45$ and $p=0.98$, respectively). Our cohort consisted of 46 infants with documented cCMV. Initiation or continuation of maternal treatment after detection of CMV-DNA in amniotic fluid did not affect outcome ($p=0.75$). Abnormal head U/S at birth correlated with hearing loss ($p=0.006$). However, when symptomatic newborns were excluded, head US alone could not predict hearing deficit in otherwise asymptomatic newborns ($p=0.37$). Antiviral treatment of infants (18/46 infants) was the only factor clearly shown to improve outcome ($p<0.001$).

Conclusion: Our study revealed the need of new biomarkers to better identify which asymptomatic infants with cCMV would benefit from antiviral treatment.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-010

Study of Cytomegalovirus Seroprevalence in the Czech Republic Population

Vlasta Stepanova, Miroslav Fajfr

University Hospital and Faculty of Medicine of Charles University, Dept. of virology of Inst. Clin. Microbiol., HRADEC KRALOVE, Czech Republic

Background. Cytomegalovirus (CMV) is a leading cause of congenital infection and disease including hearing loss and mental retardation. There is no recent official representative CMV seroprevalence survey of the Czech Republic (CZ) population. Last official CMV seroprevalence survey in CZ was performed by National Institute of Public Health in 1996. The aim of our work was to determine CMV IgG seropositivity in patients of all age groups that are routinely examined in our laboratory. As there are no

recent official data we tried to estimate suspected overall CMV seroprevalence.

Materials and methods. 4687 patients aged 0 to 80+ (2248 males and 2469 females) serum samples were tested CMV IgG (mostly with CMV IgM and selectively CMV IgG avidity) in the routine laboratory praxis using CMV IgG, Architect, Abbott assay in 2011 to 2014. CMV IgG seropositivity percentage was counted in the five-year age groups.

Results. Opposite to 45% CMV IgG seropositivity in 1996 survey we determined CMV IgG in females of age of 6-9 in 37,8%, in age 31-40 in 65,2% while 80% in 1996. Male CMV IgG seropositivity was even lower, 35,3% and 55,9%. The age group of 71-80 reached 91,2% in females and 75,6% in males in recent study while 95% and 90% in 1996. The number of CMV primary infections has increased in age of 20 to 40.

Conclusion. Our study of CMV IgG seropositivity may not be regarded as a representative seroprevalence study but according to our results we estimate that the real CMV seroprevalence in healthy population is even lower. Among women of childbearing age the CMV IgG seropositivity within 20 years has decreased. Seronegative women of childbearing years are more susceptible to primary CMV infection and prevention programs to prevent congenital CMV infection in their children must be the aim of public health intervention.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-015

Congenital human herpesvirus 6 (HHV-6) infection: birth prevalence, persistence and frequency of viral variants in saliva.

Aparecida Yulie Yamamoto, Carla Bertolini Frigori, Luis Tadeu Figueiredo, Glauciane Garcia Figueiredo, Léa Maria Maciel, Marisa Marcia Mussi-Pinhata
Ribeirão Preto Medical School, University of São Paulo, Department of Pediatrics, RIBEIRÃO PRETO, Brazil

Background: Unlike cytomegalovirus (CMV), the most frequent known cause of congenital viral infection, data on congenital Human Herpesvirus 6 (HHV-6) are scarce. We aimed to determine the birth prevalence of congenital HHV-6 infection and verify the frequency and persistence of viral variants in the saliva of congenitally infected infants.

Study design: Saliva and blood spots from 2597 newborns were collected in the first week of life. Congenital HHV-6 infection was defined by viral DNA detection in saliva and blood spots with a PCR assay. HHV-6A and HHV-6B variants were identified on the basis of differences in nucleotide sequences. Subsequent saliva and urine specimens were obtained from infected infants to verify the persistence of viral DNA.

Results: Congenital HHV-6 infection was confirmed in 10/2597 infants, a birth prevalence rate of 0.4%(CI95%: 0.2-0.7). With one exception, all infected newborn infants had no clinical signs suggestive of congenital infection. The presence of viral DNA in saliva and blood was observed in all infected infants. Viruria was detected in only 6(60%) of these infants. Six of the ten infants were infected with the variant HHV-6B, and the remaining four were infected with the variant HHV-6A. In 2587 infants, HHV-6-DNA was not found in any obtained specimens. The same HHV-6 variant was identified in saliva, blood spot and urine at birth and follow-up in each infant. Persistence of HHV-6 shedding in saliva and urine was verified from birth to a median of 15 months.

Conclusions:The HHV-6 prevalence rate seems to be lower to that reported for congenital CMV infection in the same population. Both HHV-6 variants A and B can be transmitted from mother to fetus. As CMV, infants with congenital HHV-6 infection had prolonged viral shedding in saliva and urine. Saliva but not urine is useful for detection of congenital HHV-6 infection.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
A-PP-017

Risk of Cytomegalovirus transmission with IgG avidity in the grey zone during pregnancy and the impact of Hyperimmune Globulin treatment

Kassiani Kekkou, Dimitra Kavatha, Maria Tsilika, Sofia Kanavou, Lambrini Galani, Dimitra Dimopoulou, Lampros Mariolis, Efthymia Alexopoulou, Vassiliki Papaevangelou, Anastasia Antoniadou
UNIVERSITY HOSPITAL OF ATHENS „ ATTIKO„ , ATHENS, Greece

Background .Screening of pregnant women for Cytomegalovirus (CMV) infection is common practice in Europe and management of pregnant women with acute CMV infection (seroconversion or low IgG avidity titers) remains a challenge. In our centre we offer treatment with CMV Hyperimmune Globulin (HIG) in women with acute CMV infection during pregnancy. Interpretation and management of women with IgG avidity titers in the grey zone is difficult since preexisting maternal immunity(high IgG avidity) substantially reduces, but does not eliminate the risk of fetal infection.

Patients and Methods.Pregnant women referred to our outpatient clinic for infections during pregnancy with a pregnancy age ≤ 25 weeks, positive IgG, IgM against CMV and IgG avidity in the grey zone were prospectively followed.CMV HIG was offered and follow-up included U/S, amniocentesis for virus detection and MRI if appropriate.

Results.67 women(mean age 31, mean pregnancy age 13 weeks, 10 received HIG) were evaluable in retrospective analysis. 5 terminated pregnancy(4 unrelated to CMV and 1 because of positive amniotic fluid) and 63 babies were born asymptomatic (2 with congenital CMV infection) and remained so at 2 years follow-up. The overall transmission rate was 4.6% (3.6% vs 10% for those receiving or not HIG). Despite a RR=3.8 (95% confidence limits 0.3-37) for CMV transmission without HIG, this was not statistically significant ($p=0.3$, Fisher's exact test, SAS 9.4)

Conclusions.Transmission of CMV infection during pregnancy when the IgG avidity is in the grey zone is higher compared to recurrent infection. More powered studies are needed to prove a significant reduction in transmission using HIG

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
A-PP-021

Cytomegalovirus Seropositivity and Adverse Pregnancy Outcomes in an Urban Cohort

Karen Fowler, Shannon Ross, Alan Tita, Suresh Boppana, William Britt
University of Alabama at Birmingham, Pediatrics, BIRMINGHAM, United States of America

Background: The effect of CMV on adverse pregnancy outcomes in CMV immune populations has not been fully elucidated.

Objective: To examine the association between CMV and adverse pregnancy outcomes

Methods: CMV serostatus was determined during pregnancy or at delivery for 1,079 women who delivered between October 2015 and December 2016. Maternal prenatal and delivery records were abstracted for demographic, medical, and pregnancy complications such as hypertensive diseases, diabetes mellitus, preterm labor, and premature rupture of membranes. Also, collected were pregnancy outcomes of interest including preterm birth (PTB, < 37 gestational weeks), low birth weight (LBW, < 2500 g), intrauterine growth retardation (IUGR), and chorioamnionitis.

Results: The cohort was 68% Black, 27% White, and 5% Hispanic and the mean maternal age was 24 (± 4.3) years. Prenatal care for 72% of the women was provided by the local health department and 84% had public insurance for their hospital stay. CMV seropositivity differed with race, where 90% of Hispanic women, 79% of Black women, and 55% of White women were CMV seropositive at delivery. CMV seropositive and CMV seronegative women did not differ with respect to PTB (8.1% vs. 7.0%) and LBW (7.7% vs 7.3%). However, IUGR was more likely to occur in CMV seropositive women (5.9%) than CMV seronegative women (2.2%, $p=0.01$). Also, chorioamnionitis occurred more often in CMV seropositive women (3.4%) than CMV negative women (1.1%, $p=0.05$). After adjusting for race and socioeconomic

status (SES), CMV positive women were at a 2.4 fold increased risk of IUGR (95%CI 1.0-5.7) and 3.5 fold increased risk for chorioamnionitis (95%CI, 1.0-11.9) compared to CMV negative women.

Conclusion: CMV seropositivity is associated with IUGR and chorioamnionitis in this CMV immune cohort. Further study is needed to confirm this association and the mechanisms underlying the increased risk for adverse pregnancy outcomes in CMV seropositive women.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-006

Viral Loads of Different Specimens in Congenital Cytomegalovirus Infection from Highly Immune Population

Chengbin Wang¹, Chengbin Wang¹, Shiwen Wang², Aiqiang Xu³, Xiaofang Wang², Tongzhan Wang³, Wenqiang Zhang³, Xiaolin Liu³, Haiyan Wang³, Tatiana Lanzieri¹, Minal Amin¹, Stephanie Bialek¹, Sheila Dollard¹

¹Centers for Disease Control and Prevention, ATLANTA, United States of America

²Chinese Center for Disease Control and Prevention, BEIJING, China

³Shandong Provincial Center for Disease Control and Prevention, JINAN, China

Background: Congenital cytomegalovirus (CMV) infection is the leading viral cause of birth defects and developmental disabilities, but viral loads in various specimen types have not yet been fully examined.

Methods: Wet saliva, dried saliva, dried blood spots (DBS), and cord blood were collected from newborns from 6 hospitals of Shandong Province during March 2011-December 2015. The wet saliva, dried saliva, and DBS specimens were tested with real-time PCR for CMV virus; PCR testing of cord blood specimens was conducted on 100 randomly selected cord blood samples and among those with positive CMV testing results on saliva or DBS.

Results: A total of 13184, 21509, 3995, and 135 wet saliva, dried saliva, DBS, and cord blood specimens were tested, with 90 (0.7%), 170 (0.8%), 14 (0.4), and 13 (9.6%) found to be CMV positive, respectively. The mean viral loads in wet and dried saliva (683,831 copies/mL and 565,804 copies/mL, respectively) were significantly higher than the mean viral loads of DBS and cord blood (2,732 copies/mL and 2,042 copies/mL, $P < 0.001$). There was no difference in mean viral loads in wet and dried saliva ($P = 0.75$) or DBS and cord blood ($P = 0.59$). The viral loads of both wet and dried saliva were higher among those with positive DBS or cord specimens than those with negative DBS and cord specimens (1,109,467 vs. 28,524 copies/mL, $P = 0.02$ for wet saliva; and 24,776,291 vs. 203,322 copies/mL, $P = 0.03$, for dried saliva, respectively), but not associated with presence of symptoms at birth.

Conclusions: Saliva specimens have higher viral load than blood specimens from infants with congenital CMV infection in a highly immune population, suggesting that saliva may provide higher sensitivity for detecting congenital infection. However, the sensitivity of specimen type for identifying children who may develop sequelae from CMV has not yet been determined.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-013

Congenital CMV in audiology clinics and childcare - opportunities for diagnosis and prevention using existing resources

William Rawlinson¹, Pamela Palasanthiran¹, Monica Wilkinson², Beverley Hall¹, Laila Al Yazidi², Carolyn Cottier², Wendy VanZuijlen¹

¹University of NSW and NSW Health Pathology, Serology and Virology Division, RANDWICK, Australia

²Sydney Children's Hospital Network, RANDWICK, Australia

Background: We identified congenital CMV (cCMV) in infants referred for audiology after failed UNHS with neurological hearing loss (SNHL), and in childcare centres to identify possible routes of intervention. We used existing resources with collaboration and rapid notification to develop an algorithm for timely testing for CMV in all infants with SNHL.

Methods: CMV testing of urine +/- saliva were offered and infants with CMV detected by PCR at ≤ 30 days of age in urine/saliva, were diagnosed cCMV then followed for counselling and treatment. Infants >30 days PCR positive were diagnosed as cCMV if they had positive newborn screen card or other laboratory or clinical features of cCMV.

We simultaneously sampled CMV excretion in 130 nasal samples collected from 20 childcare staff of two centres over 5 weeks, with PCR of nasal and skin swabs.

Results: A total of 1520 children failing UNHS were referred for audiological testing. 30% (469) infants had permanent hearing loss confirmed of whom 308 were offered CMV testing, 10 declined testing. There were 123 had audiology by ≤ 21 days, and 203 by ≤ 30 days, of whom 195 were tested for CMV. CCMV was diagnosed in 10 infants (9 urine, 6 saliva, urine + saliva in 7), including 1 positive NBSC). In childcare there were 8/130 carers CMV DNA positive, a CMV excretion rate of 35% in staff.

Conclusion: This program of testing identified $\sim 6\%$ of congenital CMV in a large cohort who failed UNHS and had permanent SNHL confirmed. It did not require significantly different assets to those already existing in the tertiary referral paediatric centre, and provided useful and timely information for clinical and audiological follow up.

Increased awareness of childcare CMV infection among parents and healthcare providers is necessary to minimise CMV acquisition during pregnancy and subsequently congenital CMV infection.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-018

Contamination and peculiar cases during a congenital cytomegalovirus neonatal screening program: a plea for decentralized screening

Jos Van Acker¹, Suzanne Smit², Elke Vanlaere¹, Anne-Marie Van den Abeele¹, Inge Dierickx¹, Veerle Staelens¹, Tom Vercruysse¹, Charlotte Verfaillie¹

¹AZ Sint-Lucas, Laboratory of Clinical Microbiology, GHENT, Belgium

²Ghent University, Department of Virology, Faculty of Veterinary Medicine, MERELBEKE, Belgium

Background: There is much debate about if and how screening for congenital Cytomegalovirus (CMV) should be organized. We try to demonstrate the impact on patient care of universal neonatal screening in our hospital.

Material/methods: From February 2015 a universal neonatal CMV screening program was implemented in our hospital. Saliva of the newborn is collected during the first postpartum consult by the pediatrician using Eswab (Copan, Italy) and is analyzed for CMV by an in house PCR. In case of positive saliva testing, CMV presence is confirmed by performing the same PCR on a urine sample of the newborn. Positive PCR results are also compared with CMV serology of the newborn's mother. Data from 2016 were analyzed.

Results: 2254/2291 (98.4%) of all newborns were screened. 42 (1.8%) babies were CMV positive. 31 (74%) appeared to be false positive, 11 (26%, PPV) were considered true cases of congenital CMV. Two cases, that would not have been detected without the screening program, caught the eye. The first case was a symptomatic baby with combined congenital CMV and Toxoplasmosis with maternal CMV infection before pregnancy and a *Toxoplasma gondii* seroconversion between 26 and 34 weeks of pregnancy. The second case was a baby with congenital CMV infection, asymptomatic at birth, with maternal second trimester CMV seroconversion during the previous pregnancy in 2013 without transmission at that time.

Conclusions: In our neonatal CMV screening program we found true positive and contamination rates of respectively 0.5 and 1.4%. As confirmation of positive saliva CMV screening results is necessary by analysis of an independent urine sample or serology of the mother, a decentralized approach of universal CMV screening may be more efficient. Two intriguing cases of congenital CMV show the

surplus value of close patient follow-up in this screening program on therapeutic choices and patient counseling.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-019

Congenital CMV infection and the occurrence of cystic periventricular leukomalacia

Josine In 't Veld, Philip Elders, Moniek Van de Loo, Katja Wolthers, Dasja Pajkrt
Emma Children's Hospital, Academic Medical Center Amsterdam, Department of Pediatric Infectious Diseases, AMSTERDAM, Netherlands

Periventricular leukomalacia (PVL) is a leading form of brain injury in the premature infant and can be potentiated by infection and inflammation. The nature of pathology in PVL has been found to be similar to that of congenital *Cytomegalovirus* (CMV) infection. Therefore, we evaluated whether there is an increased prevalence of congenital CMV infection in neonates that developed cystic PVL in a retrospective multi-center study in the Netherlands. We included a cohort of 35 preterm and term neonates with cystic PVL admitted from March 2011 to March 2016 in different tertiary Neonatology Intensive Care Units in the Netherlands. CMV status was determined by performing PCR on Guthriecards or by a previously established negative CMV status. No children with cystic PVL suffered from a congenital CMV infection. Our results show no association between congenital CMV infection and cystic PVL.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-022

Challenges of a universal newborn screening for congenital cytomegalovirus infections by saliva specimens

Emmanouela Dimitrakopoulou¹, Katrin Neumann¹, Khalid Shahada², Norbert Teig¹, Klaus Überla³, Thomas Lücke¹, Dariusz Michna⁴, Peter Kern¹, Stefan Niesert⁴, Angela Nagel³, Hans-Joachim Trampisch¹

¹Ruhr University Bochum, Dept. of Phoniatrics and Pediatric Audiology, BOCHUM, Germany

²Hamad Medical Corporation, Audiology and Balance Center, DOHA, Qatar

³University Erlangen, Inst. of Clinical and Molecular Virology, ERLANGEN, Germany

⁴St. Elisabeth-Hospital Essen, Children's Hospital, ESSEN, Germany

The reasonableness and feasibility of a universal newborn screening for congenital cytomegalovirus (cCMV) infections has been ascertained through several studies. However, the implementation of such a screening comes along with a variety of practical challenges.

So far 3242 infants born in two German clinics have undergone an unselected screening for cCMV in the framework of an international study running in Qatar and Germany*. Saliva specimens were collected during the routine newborn metabolic screening and analyzed by real-time PCR. cCMV positive infants were enrolled in a 6-year audiological, neuropediatric, ophthalmological and radiological follow-up-program.

Apart from a high screening validity (specificity 99.7%, sensitivity 100%), the acceptance rate of the parents varied from 57% to 85% across clinics and the lost-to follow-up rate of infected children was 25%.

The preliminary program outcome suggests that a universal neonatal cCMV screening needs to fulfill

several quality criteria. A sufficient coverage rate has to be achieved in order to make the program effective. The acceptance of the screening by both the parents and the screening staff has to be ensured by education of parents, already during pregnancy, and by training and supervision of the screening staff (nurses, midwives, obstetricians, pediatricians). If a baby has failed the screening the parents need to be provided with addresses and an agenda of a confirmation diagnostics and—if an infection is confirmed—of a follow-up program. A high adherence of parents to the follow-up program needs their continued information and is particularly challenging when children are asymptomatic at birth. The follow-up program requires a close interdisciplinary cooperation and good logistics between audiologists, pediatricians, ophthalmologists, and labs. A data collection in a screening database is required in order to enable both a completeness tracking and a tracking of the babies who have failed the screening.

*Funded by the QNRF (NPRP 7-1845-3-480).

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-025

Detection of CMV in urine and mother's milk samples among the babies in neonatal ICU in Motol University Hospital.

Petr Hubacek

Motol University Hospital, Department of Medical Microbiology, PRAGUE, Czech Republic

Interaction and manipulation of the CMV with the immune system of the baby is involved in majority of the cCMV complications, there is a question how CMV influence the post-natal development of the pre-term babies. Aim of our study was to find out the frequency of CMV among the babies at Neonatal Intensive care Unit (NICU).

Between July 2016 and January 2017, we obtained 96 samples of mother's milk (MM) from 69 mothers and 117 urine/urine containing surgical swabs after inserting into the nappy from 83 newborns (39 girls and 44 boys) shortly after the delivery with median 1 day of age in urine samples (range 0-104 days) and 5 days of age in MM (range 0-53 days). There were 14 twins in the cohort. CMV DNA detection was performed by PCR and validity of the detection was controlled by detection of the human albumine gene in the sample.

Validity of the testing failed in 9 urine samples. CMV was detected in 4 urine samples from 3 infants (at the age of 38, 48 and 59 days) and 30 MM samples from 20 babies (16 mothers – 23.2%; median at the first positive sample was 10 days; range 1-35). In 7 mothers, CMV was detected in MM repeatedly (longest detected period 62 days). In two infants CMV detection preceded CMV in MM in detection urine for 35 and 53 days.

Despite the freezing of the MM before feeding the babies at NICU, we detected CMV in approx. 4% of the tested neonates. Recently, we are working on the genotyping of CMV both from MM and urine samples to prove the infection from MM or infection from another source and analysing the clinical features and complications observed in the infants.

Supported by the project for conceptual development of research organization 00064203.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-026

Infants diagnosed with congenital CMV after NHS refer: a variety of signs in infected infants without clinical signs at birth

Wim Soede, F.A.J. Schornagel, L.G. Rusman, A.C.M. Kroes, A.M. Oudesluys-Murphy, A.C.T.M. Vossen

LUMC, LEIDEN, Netherlands

The majority of infants with congenital CMV (cCMV) with long term sequelae (hearing loss, visual impairment, developmental delay) are born without clinically apparent disease in the neonatal period. If CMV diagnostics is only performed in symptomatic infants many infants at risk of developing sequelae will not be diagnosed. The CONCERT study gives more insight into sequelae in infants without clinically apparent disease, referred after neonatal hearing screening.

Parents of infants who failed neonatal hearing screening (NHS) in the Netherlands were invited to participate in the CONCERT study (NCT02005822). Dried blood spots, obtained within 5 days of birth, were tested for CMV. A physical examination was performed at the age of 2-3 months. Audiological examination was performed before 2 months. Brain ultrasound performed upon consulting a Pediatrician.

1378 infants who failed NHS were tested for cCMV, and 59 (4,3%) tested positive. At the first hearing evaluation bilateral hearing loss was confirmed in 24, unilateral HL in 28 and 7 infants had normal hearing. Physical examination of 37 infants revealed 9 with a head circumference \leq -2 SD. Laboratory findings were complete for 34 infants and revealed 6 with neutrophil count $<1,0 \times 10^9/L$ and 3 with elevated liver transaminases (ASAT ≥ 89 U/L ; ALAT ≥ 60 U/L). None of these infants required additional treatment. With brain ultrasound in 33 infants, abnormalities were found in 21. MRI examination was performed in 15 infants and revealed abnormalities in 8 infants ranging from mild defects to polymicrogyria.

These data emphasize the risk for developing hearing loss without apparent clinical disease. Also in many infants abnormalities were found with brain ultrasound and MRI examination. Considering that infants diagnosed with cCMV receive additional care (extra hearing evaluations, brain ultrasound, medical support from a pediatrician and ophthalmologic evaluation) CMV diagnostics should be offered to all infants with a NHS refer.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-011

Loosing Ground: Awareness of Congenital Cytomegalovirus in the United States

Sara Doutré¹, Karl White¹, Tyson Barrett¹, Janelle Greenlee²

¹National Center for Hearing Assessment and Management, Utah State University, LOGAN, UTAH, United States of America

²National CMV Foundation, SARASOTA, CALIFORNIA, United States of America

One in 150 infants is born with cytomegalovirus (CMV) and one in 750 will have lifelong disabilities due to congenital CMV. Even though congenital CMV is the leading viral cause of congenital disabilities and the leading non-genetic cause of childhood hearing loss, most adults have never heard of it. This study analyzed data from the 2015 and 2016 United States HealthStyles™ surveys and compared them to data from previous studies. Most recent data show an awareness rate of 7% for US adults (5% for men and 9% for women), a statistically significant decrease from 2005 and 2010 studies. Multiple logistical regression analyses demonstrate that predictors of awareness include gender and education level. The presence of a child ages 0-5 in the household does not increase the chance that an adult in the household is aware of CMV. Latent class analyses show several classes with varying knowledge of conditions impacting newborns. For all classes, including one class with high knowledge of ten conditions, nine with lower prevalence than congenital CMV, CMV is the least known condition. Results show that even the highest-educated, highest socioeconomic group is not aware of congenital CMV. CMV presents a large public health burden and further research needs to be focused on awareness and prevention of the negative sequela associated with congenital CMV.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-012

Raising Awareness of Congenital CMV through Legislative Advocacy: The Expansion of CMV Laws in the United States

Sara Doutre¹, Kristen Spytek²

¹National Center for Hearing Assessment and Management, Utah State University, LOGAN, UTAH, United States of America

²National CMV Foundation, TAMPA, FLORIDA, United States of America

Thank you to the efforts of parent and professional advocates, several US state governments now play a vital role in congenital cytomegalovirus (CMV) research, treatment, and diagnostic testing for babies with congenital CMV. State legislatures mandate screening or testing for CMV, provide funds for research and awareness programs, and provide incentive to government agencies to raise awareness of congenital CMV. Since 2013, parent and professional advocates in the United States have successfully brought attention to CMV through legislation passed by individual state legislatures. As of January 1, 2017, five states have laws in effect requiring education programs, CMV testing for specific infants, or both. An additional eight states have proposed legislation including one state, Maine, with a legislative bill in progress that will require universal newborn CMV screening. This presentation discusses the prerequisite steps for proposing legislation: building a team of necessary advocacy and professional partners, garnering support from agencies and organizations, and organizing and coaching parent and other advocates to have the maximum influence. We also discuss the barriers to CMV public health policy faced by advocates, professionals, and policy makers. Finally, the presentation summarizes the various approaches in each law for educating professionals and the general public about CMV and for testing newborns. The continued interest in and success of advocates in passing state legislation in the US has multiple implications for professionals and effective implementation requires collaboration. Next steps for parent and professional advocates include submission of congenital CMV to the recommended universal screening panel in the United States and continued efforts to demonstrate the feasibility of screening and the effectiveness of awareness and prevention campaigns.

270 - Poster Presentation Track A

Date: 01/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

A-PP-027

Prevalence of Cytomegalovirus Strains in the United States: the National Health and Nutrition Examination Surveys, 1999-2004

Chengbin Wang¹, Minal Amin¹, Adam Wharton², Manisha Patel², Sheila Dollard¹

¹Centers for Disease Control and Prevention, ATLANTA, United States of America

²CDC, ATLANTA, United States of America

Background Genetic variations between strains of cytomegalovirus (CMV) genetic variations may be associated with various clinical outcomes. However, the prevalence of CMV strains in the US has not been examined.

Methods Urine specimens from 537 CMV IgG+ participants from the 1999-2004 National Health and Nutrition Examination Surveys aged 6-49 years and from three racial/ethnic groups (non-Hispanic white, non-Hispanic black, and Hispanic [Mexican-American]) were tested by polymerase chain reaction to determine the CMV glycoprotein B (gB) and glycoprotein H (gH) genotypes.

Results Among the four gB strains from 205 subjects with gB data available, gB1 had the highest prevalence (51.2%), followed by gB2 (27.8%), gB3 (21.0%) and gB4 (13.7%). Among the two gH strains from 251 subjects with gH data available, the prevalence was 48.2% for gH1 and 59.0% for gH2. Those with gB2 strain had higher viral loads than those without (3.3 vs. 3.1 log₁₀ copies/mL, P=0.03) and

foreign-born residents had higher gB4 prevalence than US-born residents (22.0% vs. 11.6%, $P=0.03$). A total of 43 (14.9%) subjects had more than one strain detected (mixed infection) in 288 subjects with genetic data available. Mixed infection was more likely in foreign-born versus US-born residents (26.3% vs. 12.1%, $P=0.004$) and in females compared to males (17.4% vs. 12.0%, $P=0.01$).

Conclusions This study provides national data on the molecular epidemiology of CMV strains in the US and identifies strain-associated variations in geographic distribution and pathogenesis. The high prevalence of mixed infection further verifies the occurrence of reinfection and its contribution to the overall CMV disease burden. Further studies evaluating the clinical significance of genetic variability of CMV strains are needed.

280 - Session C.01: Clinical Practice, Problems & Solutions

Date: 01/05/2017

Time: 17:00 - 18:15 hrs

Invited presentation

C-INV-001

CMV infection, immunological ageing and clinical consequences in the immune comprised host

Michiel Betjes

Erasmus Medical Center, Nephrology and Transplantation, ROTTERDAM, Netherlands

Cytomegalovirus has a major impact on the composition of the circulating T cell population. Several aspects, such as decreased telomere length, an increase in more differentiated CD4 and CD8 T cells and T cell receptor oligoclonality in individuals with CMV latency, mimic immunological ageing. Particular in individuals with chronic inflammatory conditions the impact of CMV latency appears to be most pronounced. Severe loss of renal function leading to the need for renal replacement therapy (dialysis or kidney transplantation) is characterized by a pro-inflammatory environment with a decreased functionality of the adaptive immune response. This is clinically relevant as these patients have more infections, respond poorly to vaccination and have an increased susceptibility for viral-associated tumors. Therefore, renal failure is a clinical model of the effects of chronic inflammation on the immune system. Several characteristics of the peripheral T cells like thymic output, telomere length, T cells differentiation status and T cell receptor clonality strongly suggest premature immunological ageing. In large patient cohorts the role of CMV latency was further delineated. The impact of CMV latency on the extent of immunological ageing in patients with renal failure is discrete and not evident for every parameter of immunological ageing. In addition, it is an age-dependent phenomenon affecting in particular the premature immunological ageing in elderly patients. The potential pathogenesis and clinical consequences will be discussed.

280 - Session C.01: Clinical Practice, Problems & Solutions

Date: 01/05/2017

Time: 17:00 - 18:15 hrs

BOTH ORAL & POSTER PRESENTATION

C-OP-001

Challenges and opportunities in sequencing complete human cytomegalovirus genomes directly from clinical material

Nicolas Suarez¹, Gavin Wilkie¹, Maurizio Zavattoni², Milena Furione², Maria Revello³, Daniele Lilleri⁴, Mauro Stronati⁵, Giuseppina Lombardi⁵, Fausto Baldanti², Petr Hubáček⁶, Elias Hage⁷, Tina Ganzenmueller⁷, Thomas Schulz⁷, Giuseppe Gerna⁴, Andrew Davison¹

¹MRC-University of Glasgow Centre for Virus Research, GLASGOW, United Kingdom

²Molecular Virology Unit, Microbiology and Virology Department, Fondazione IRCCS, PAVIA, Italy

³Departments of Obstetrics and Gynecology, Fondazione IRCCS Policlinico San Matte, PAVIA, Italy

⁴Experimental Research Laboratories-Transplantation Area, Fondazione IRCCS Policlinico San M, PAVIA, Italy

⁵Neonatology and Neonatal Intensive Care Unit, Fondazione IRCCS Policlinico San M, PAVIA, Italy

⁶Department of Medical Microbiology, Motol University Hospital, PRAGUE, Czech Republic

Human cytomegalovirus (HCMV) can be a serious problem in congenital or transplant-acquired infections, and also in people infected with HIV. Infections involving multiple strains have been linked to poor clinical outcome in immunocompromised patients, and some studies in congenital cases reflect a similar linkage. The viral genome is 236 kbp in size and has several long-recognised features that present challenges to genomic studies. These include the existence of hypervariable genes, the presence of gene-disrupting mutations, the prevalence of infections involving multiple strains, an evolutionary history characterised by extensive recombination, and a propensity to mutate when isolated in cell culture. As a result, meaningful studies of pathogenesis are bound to include analysing strains directly from clinical material in the context of a sound understanding of the number of strains present, as failure to monitor this aspect of infection may lead to the derivation of genome sequences representing artefactual chimaeras and to overestimates of the intrahost diversity of the individual strains present. By using modified Illumina sequencing library preparation and target enrichment techniques coupled to a bioinformatics pipeline for sequence assembly, we have determined the sequences of many HCMV genomes from such material and deposited them in GenBank. We have incorporated in this pipeline a means of assessing whether each library is sufficiently diverse to represent the sample adequately, as well as a genotyping approach that utilises several hypervariable genes to identify distinct viral strains and estimate their proportions. We have thus far applied this pipeline to 158 samples from 60 patients, the majority from transplant situations. Importantly, this approach has detected signs of intrahost recombination in some longitudinal samples involving multiple strain infections.

280 - Session C.01: Clinical Practice, Problems & Solutions

Date: 01/05/2017

Time: 17:00 - 18:15 hrs

BOTH ORAL & POSTER PRESENTATION

C-OP-002

Clinical validation of T-Track[®] CMV to assess the functionality of CMV-specific cell-mediated immunity in kidney transplant recipients

Anne Rasclé¹, Bernhard Banas², Dominik Steubl³, Lutz Renders³, Dominik Chittka², Miriam Banas², Thomas Wekerle⁴, Martina Koch⁵, Oliver Witzke⁶, Anja Muehlfeld⁷, Claudia Sommerer⁸, Antje Habicht⁹, Christian Hugo¹⁰, Thomas Huenig¹¹, Monika Lindemann¹², Traudel Schmidt¹, Sascha Barabas¹, Ludwig Deml¹, Ralf Wagner¹³, Bernhard Kraemer¹⁴, Bernd Krueger¹⁴

¹Lophius Biosciences, REGENSBURG, Germany

²University Medical Center Regensburg, Nephrology, REGENSBURG, Germany

³Klinikum rechts der Isar Technical University Munich, MUNICH, Germany

⁴Medical University of Vienna, Surgery, VIENNA, Austria

⁵University Medical Center Hamburg-Eppendorf, HAMBURG-EPPENDORF, Germany

⁶University Hospital Essen, Departments of Nephrology and of Infect, ESSEN, Germany

⁷Uniklinik RWTH Aachen, AACHEN, Germany

⁸University Hospital Heidelberg, Nephrology, HEILDELBERG, Germany

⁹Ludwig-Maximilians-University Medical Center Munich, MUNICH, Germany

¹⁰Carl Gustav Carus University Medical Center Dresden, DRESDEN, Germany

¹¹University Medical Center Wuerzburg, WUERZBURG, Germany

¹²Institute for Transfusion Medicine University Hospital Essen, ESSEN, Germany

¹³University Hospital Regensburg, Clinical Microbiology and Hygiene, REGENSBURG, Germany

¹⁴University Medical Center Mannheim, MANNHEIM, Germany

Impaired cytomegalovirus (CMV)-specific cell-mediated immunity (CMV-CMI) is a major cause of CMV reactivation and associated complications in solid-organ transplantation. Reliably assessing CMV-CMI is desirable to individually adjust antiviral and immunosuppressive therapy. The aim of this study was to evaluate the suitability of T-Track[®] CMV, a novel immune-monitoring IFN- γ ELISpot assay, to survey CMV-CMI in renal transplant recipients.

A prospective, longitudinal, multicenter study was conducted in a cohort of 96 intermediate risk (D-/R+, D+R+) renal transplant recipients under preemptive antiviral strategy, over 6 months post-transplantation. T-Track[®] CMV was used to quantify CMV-reactive effector cells in response to T-

activated[®] pp65 and IE-1 proteins. CMV viral load (quantitative PCR or pp65 antigenemia) and related complications, opportunistic infections and graft function were also monitored. 95% and 88-92% T-Track[®] CMV assays were positive pre- and post-transplantation, respectively. CMV-specific response was reduced following immunosuppressive treatment and increased in patients with graft rejection, indicating the ability of the ELISpot assay to monitor patients' immunosuppressive state. Interestingly, median pp65-induced SFC level was 9-fold higher in patients with self-clearing viral load compared to antivirally-treated patients (534 vs. 60 SFC/200,000 cells; n=10; p<0.001) prior to first detection of viral load, suggesting that the response to pp65 represents a potential immunocompetence marker. Altogether, T-Track[®] CMV is a highly sensitive IFN- γ ELISpot assay, suitable for the immune monitoring of renal transplant recipients, and with a potential use for the risk assessment of CMV-related clinical complications.

280 - Session C.01: Clinical Practice, Problems & Solutions

Date: 01/05/2017

Time: 17:00 - 18:15 hrs

BOTH ORAL & POSTER PRESENTATION

C-OP-003

CYTOMEGALOVIRUS-SPECIFIC CD8+ T-CELL IMMUNITY IN NEONATES WITH CONGENITAL INFECTION: CORRELATION WITH CLINICAL AND LABORATORY FINDINGS

Concetta Marsico¹, Maria Grazia Capretti¹, Francesca Nanni¹, Angela Chiereghin², Liliana Gabrielli², Gabriele Turello², Diego Squarzone², Giacomo Faldella¹, Tiziana Lazzarotto²

¹Operative Unit of Neonatology, St. Orsola-Malpighi Polyclinic, University of Bologna, BOLOGNA, Italy

²OU Microbiology, Laboratory of Virology, St. Orsola-Malpighi Polyclinic, BOLOGNA, Italy

Background: Cytomegalovirus (CMV)-specific cell-mediated immunity (CMI) plays a crucial role in controlling CMV disease. The aim of this study was to evaluate usefulness and predictive role of QuantiFERON[®]-CMV (Cellestis-Qiagen) assay, based on evaluation of IFN- γ secretion by CMV-specific CD8+T-cells, in neonates with congenital CMV (cCMV) infection.

Methods: Fifteen neonates with cCMV infection were included. Blood samples for QuantiFERON[®]-CMV assay and for viral load (VL) were obtained within the end of both second week and second month of life. For QuantiFERON[®]-CMV test blood was collected into three tubes: CMV peptide pool ("CMV"), negative ("nil") and positive ("mitogen") controls. IFN- γ (expressed as IU/mL) responses were: positive (detectable CMV-specific CMI) if [CMV-nil] \geq 0.2; negative (absent CMV-specific CMI but general T-cell responsiveness) if [CMV-nil]<0.2 and [mitogen-nil] \geq 0.5; indeterminate (absence of any CMI) if [CMV-nil]<0.2 and [mitogen-nil]<0.5. Correlations with whole blood VL, neonatal and long-term outcome were analyzed.

Results: Median age at QuantiFERON[®]-CMV samples were 11 (range 4-15) and 39 (range 28-62) days respectively. 7/15 (47%) infants had positive QuantiFERON[®]-CMV results at both evaluations and were asymptomatic. 6/15 (40%) infants had negative QuantiFERON[®]-CMV results at both evaluations and all but one were symptomatic (neuroimaging abnormalities 3/5, SNHL 2/5, disseminated disease 1/5). 1/15 (6.5%) had a negative first QuantiFERON[®]-CMV test but a second positive result, and was asymptomatic. 1/15 (6.5%) infants had indeterminate results at both evaluations and was symptomatic at birth. QuantiFERON[®]-CMV negative/indeterminate results correlated with symptomatic infection at both time-points ($p=0.007$ and $p=0.001$ respectively). Mean blood VL at first sample was comparable in infants with positive and with negative/indeterminate QuantiFERON[®]-CMV results ($p=0.68$); at second evaluation subjects with negative/indeterminate QuantiFERON[®]-CMV results had significant higher VL as compared with those with positive results ($p=0.02$).

Conclusion: QuantiFERON[®]-CMV assay seems to reliably evaluate CMV-specific CMI in cCMV infection. The presence of CMV-specific CMI seems to correlate with asymptomatic infection at birth and during follow-up, while its lack correlates with symptomatic disease and with worse control of viral replication. Further studies are needed to confirm the prognostic value of this test performed during neonatal period.

310 - Session B.03: Virus and Host

Date: 02/05/2017

Time: 08:30 - 10:25 hrs

Keynote lecture

B-KEY-001

Using the Nonhuman Primate Model to Inform Clinical Prevention and Therapeutic Interventions Against Human CMV

Peter Barry¹, Yujuan Yue¹, William Chang¹, Alice Tarantal¹, Nancy Nguyen¹, Kimberli Schmidt¹, Diego Castillo¹, Hung Kieu¹, Louis Picker², Klaus Früh², Ellen Sparger¹

¹Center for Comparative Medicine, California National Primate Research Center, DAVIS, United States of America

²Oregon Health and Sciences University, Vaccine and Gene Therapy Institute, BEAVERTON, OR, United States of America

Despite considerable efforts spanning four decades to develop a vaccine that confers protective immunity against the devastating consequences of congenital HCMV (cCMV) infection, there is no licensed vaccine for the prevention of either primary or non-primary maternal HCMV infection. Given the absence of a licensed vaccine, there have been parallel efforts to minimize the risk of cCMV infection and sequelae based on HCMV natural history in which preconceptional immunity confers partial protection against fetal infection and disease. Therapeutic treatment of cCMV infection with HIG holds promise to limit the devastating consequences of intrauterine HCMV, but collectively, clinical results are equivocal. There are multiple uncontrollable variables associated with HIG trials that will influence outcomes and interpretations. These include (a) precise gestational timing of maternal and fetal infections, (b) differences in viral burdens in mothers and fetuses, (c) the immune specificities of HIG required to neutralize virus dissemination, (d) the timing, dose, and route of HCMV-HIG administration, and (e) the specificity of HIG relative to the infecting strain(s). The status of HIG as a prevention or therapeutic modality for cCMV is perhaps best summed up in one review, “*There is a strong need for more data on the use of HIG*” [Hamilton et al., 2014]. Given the costs and logistical complexities of sufficiently powered human trials, advances in the nonhuman primate (NHP) model of HCMV now offer clinically relevant opportunities for rigorously interrogating the protective efficacy of vaccine and HIG intervention strategies, thus informing human clinical practice. Results from NHP studies investigating (1) cross-neutralization of heterologous rhesus CMV (RhCMV) strains, (2) oral reinfection of immune animals, (3) the need for a better understanding of mucosal immunity against mucosal CMV infection, and (4) the challenges posed by the exquisite targeting of the CNS by intrauterine RhCMV will be discussed.

310 - Session B.03: Virus and Host

Date: 02/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-009

Cytomegalovirus reactivation in bone marrow transplantation: is controlling graft-versus-host disease important?

Paulo Martins¹, Siok-Keen Tey¹, Peter Fleming², Ashik Ullah¹, Rachel Kuns¹, Antiopi Varelias¹, Christopher Andoniu², Mariapia Degli-Esposti², Geoffrey Hill¹

¹QIMR Berghofer, BRISBANE, Australia

²Centre for Experimental Immunology, Lions Eye Institute, NEDLANDS, Australia

Cytomegalovirus (CMV) infection remains a significant complication after allogeneic bone marrow transplantation (BMT) and can lead to life-threatening organ damage. **Aim:** To elucidate the mechanisms of CMV reactivation in a clinically relevant model. **Method:** Mice were infected with murine CMV (MCMV) and functional latency defined as the resolution of viraemia in target organs and plasma. Latently infected mice were transplanted with Bone Marrow (BM) and T cells or T cell-depleted (TCD) BM alone to generate GVHD and non-GVHD conditions respectively. Reactivation was determined by

qPCR on plasma and plaque assays in target organs after BMT. **Results:** MCMV enters latency two months after primary infection with the resolution of viremia correlating with absence of replicating virus in organs by plaque assays. The reactivation of MCMV after BMT is GVHD-dependent: BALB/c@B6: 63% vs 17% ($p < 0.05$) with plaque-forming units detected in 54% vs 0% of livers ($p < 0.01$) and 46% vs 8% of lungs, 4 weeks post-transplant, in GVHD vs non-GVHD recipients. In a time course experiment using a LacZ reporter virus, multiple viral foci were detected early, 3 weeks post-transplant, in the liver and lungs of mice, compared to the BM and gut, suggesting they are sites of early MCMV reactivation after BMT. In the absence of GVHD, FcγR independent antibody mediated protection is sufficient to control reactivation: latently infected $\mu\text{Mt}^{-/-}$ mice, without GVHD, are highly susceptible to reactivation, 100 vs 0% ($p < 0.001$) in $\mu\text{Mt}^{-/-}$ vs WT mice, respectively. **Conclusion:** These data show that CMV reactivation in BMT is GVHD-dependent. T and NK lymphopenia in itself is insufficient to induce CMV reactivation in the presence of antibody-mediated protection.

310 - Session B.03: Virus and Host

Date: 02/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-010

Infection of the salivary gland by MCMV is dispensable for formation and persistence of salivary gland CD8⁺ TRM

Sofia Caldeira-Dantas, Corinne Smith, Thomas Furmanak, Christopher M. Snyder

Department of Immunology and Microbiology, Thomas Jefferson University, PHILADELPHIA, United States of America

Murine cytomegalovirus (MCMV) is a natural mouse herpesvirus that persists in the salivary gland, a major site of viral shedding. MCMV-specific CD8⁺ T cells develop into tissue-resident memory T cells (T_{RM}) in the salivary gland after an acute MCMV infection. T_{RM} cells develop in many tissues of the body in response to local signals. However, the requirements for T cell priming and T_{RM} formation vary by tissue, and are not defined for the salivary gland. Interestingly, recent work suggested that systemic inflammation amplifies T cell access to the salivary gland. Salivary gland infection by MCMV was associated with a transcriptional program indicative of interferon signaling and an increase in chemokines associated with T cell recruitment. Therefore, we tested whether the route of infection and infection of the gland itself modulated the recruitment and formation of salivary gland CD8⁺ T_{RM}. Altering the route of infection or depleting CD4⁺ T cells, which allows increased MCMV replication in the salivary gland, impacted the number of CD8⁺ T cells but had minimal impact on the formation of MCMV-specific T_{RM} populations. To investigate T cell recruitment and differentiation without the influence of antigen in the salivary gland, we transferred activated T cells not specific for MCMV into infected and uninfected mice. Activated T cells accumulated more rapidly in infected salivary glands, and migration experiments suggested redundancy in the chemokines used by T cells to access infected glands. Access to the gland was dependent on α₄ integrin regardless of infection. Surprisingly however, identical numbers of T cells became T_{RM} over time in infected and uninfected glands. Moreover, MCMV-specific T_{RM} did not preclude the formation of new, unrelated T_{RM} in the salivary gland. These data suggest that MCMV infection of the salivary gland have a minimal impact on CD8⁺ T_{RM} formation, despite altering the local inflammatory milieu.

310 - Session B.03: Virus and Host

Date: 02/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-011

Changes in the total cell proteome during human cytomegalovirus latency - insights into effects on latently infected monocytes

Emma Poole, Yusuf Aslam, James Wilkinson, Paul Lehner, John Sinclair, Poole Emma
University of Cambridge, CAMBRIDGE, United Kingdom

One established site of human cytomegalovirus (HCMV) latency and reactivation in vivo is in cells of the myeloid lineage. Latency is established in myeloid progenitors, such as CD34+ progenitor and CD14+ monocytes, and as these cells differentiate into dendritic cells (DCs) or macrophages, the virus reactivates. We have long been interested in latency-associated changes in myeloid cells during virus latent carriage and have now carried out an analysis of the latency-associated monocyte proteome to identify the impact of latent infection on myeloid cells. To do this, we infected primary monocytes with an SV40-GFP tagged TB40E strain of HCMV and enriched for the latently infected population by FACS sorting of GFP+ cells. The resulting cells were then subjected to a total cellular proteomic analysis. The screen was carried out in triplicate in 3 independent biological replicates. A number of cellular proteins were robustly up or down regulated and were subsequently validated by western blot. Amongst the proteins which were robustly down-regulated by latent infection were S100A8 and S100A9 which are secreted proteins known to play a role in neutrophil chemoattraction. Consistent with this, supernatants from latently infected monocytes contained less S100A8/A9 when assayed by ELISA and were less able to recruit neutrophils in transwell assays than supernatants from mock infected monocytes. These data indicate that changes associated with latent infection of CD14+ monocytes minimise recruitment of neutrophils to these latently infected cells.

310 - Session B.03: Virus and Host

Date: 02/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-012

HCMV reinfection in congenitally infected infants: an evolutionary perspective

Cornelia Pokalyuk¹, Nicholas Renzette², Kristen Irwin³, Susanne Pfeifer⁴, Laura Gibson², William Britt⁵, Aparecida Yamamoto⁶, Marisa Mussi-Pinhata⁶, Timothy Kowalik², Jeffrey Jensen⁴

¹Goethe University - Institute for Mathematics, FRANKFURT, Germany

²University of Massachusetts Medical School, WORCESTER, United States of America

³École Polytechnique Fédérale de Lausanne, LAUSANNE, Switzerland

⁴Arizona State University, TEMPE, United States of America

⁵University of Alabama Birmingham, BIRMINGHAM, United States of America

⁶University of São Paulo, SÃO PAULO, Brazil

HCMV populations in some congenitally infected infants diverge rapidly over time and between tissue compartments, while in other infants the populations remain stable. We investigate these different patterns, particularly regarding reinfection during gestation. Since the virus persists in the host after infection, a reinfected host harbors a viral population founded by virions from different infection events. As the HCMV population is highly variable, these founding virions likely differ substantially at the genomic level. Therefore, viral populations observed in hosts with HCMV reinfection may be more diverse than in those with primary infection. In pregnant women, a highly diverse viral population may pass to the fetus following maternal reinfection compared to maternal primary infection. To evaluate such scenarios, we estimated the population histories of viruses sampled from congenitally infected infants. Infant samples with HCMV likely transmitted from both pregnant women with primary and with reinfection will be highlighted. We suppose, that the relative abundance of HCMV diversity transmitted to the fetus may be a factor determining severity of disease outcome of congenitally infected infants from pregnant women with HCMV reinfection.

310 - Session B.03: Virus and Host

Date: 02/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION
B-OP-013

Control of immune ligands by members of the cytomegalovirus US12 gene family suppresses natural killer cell activation

Ceri Fielding¹, Michael Weekes², Luis Nobre², Eva Ruckova³, Gavin Wikie⁴, Joao Paulo⁵, Chiwen Chang², Nicolas Suarez⁴, James Davies¹, Robin Antrobus², Richard Stanton¹, Rebecca Aicheler¹, Hester Nichols¹, Borek Vojtesek³, John Trowsdale², Andrew Davison⁴, Steven Gygi⁵, Peter Tomasec¹, Paul Lehner², Gavin Wilkinson¹

¹Cardiff University, Infection and Immunity, CARDIFF, United Kingdom

²Cambridge University, Cambridge Institute for Medical Research, CAMBRIDGE, United Kingdom

³Masaryk Memorial Cancer Institute, Centre for Applied Molecular Oncology, BRNO, Czech Republic

⁴University of Glasgow, MRC Centre for Virus Research, GLASGOW, United Kingdom

⁵Harvard Medical School, Cell Biology, BOSTON, United States of America

The human cytomegalovirus (HCMV) US12 family consists of ten sequentially arranged genes (US12-21) with poorly characterized function. We previously observed that two US12 family members, US18 and US20, co-operate to suppress cell surface expression of the NK cell-activating ligand MICA by targeting it for lysosomal degradation (Fielding *et al.*, 2014). We now identify further novel NK cell evasion functions for four members: US12, US14, US18 and US20. Using a systematic multiplexed proteomics approach to quantify ~1,300 cell surface and ~7,200 whole cell proteins, we demonstrate that the US12 family selectively targets plasma membrane proteins and plays key roles in regulating NK ligands (e.g. MICA, MICB and B7-H6), adhesion molecules (e.g. ALCAM, EPHA2, JAM3 and CXADR) and cytokine receptors (e.g. IL6ST and TNFRSF12A). Multiple US12 family members targeted the same cellular targets, indicating some level of co-operation in their function. A proteomic analysis of HCMV-infected cells treated with a lysosomal inhibitor (leupeptin) showed the expression of most US12 family targets were rescued by leupeptin to some degree. In addition to their regulation of MICA, US18 and US20 work in concert to suppress cell surface expression of the critical NKp30 ligand B7-H6 thus inhibiting NK cell activation. The US12 gene family is therefore identified as a major new hub of immune regulation.

330 - Session C.02: Clinical Practice, Problems & Solutions

Date: 02/05/2017

Time: 10:55 - 12:55 hrs

Invited presentation

C-INV-002

Application and Quality Aspects of Molecular Methods for CMV Diagnosis and Monitoring

Rob Schuurman

UMCU, Virology, UTRECHT, Netherlands

Molecular methods are widely applied in the diagnosis and monitoring of primary and reactivating CMV infection. Qualitative molecular methods generally suffice for diagnosis of infection, whereas quantitative methods, measuring viral load, are used to monitor viral reactivation in patients upon transplantation or to detect viral rebound during antiviral treatment. Viral load monitoring in transplant patients, in particular in SCT recipients, is of pivotal importance to identify viral reactivation and to direct adequate and timely clinical decisions. CMV viral load determination has been implemented by many laboratories worldwide, either based on various CE/IVD certified based commercial methods or using so called Laboratory Developed Tests (LDT). To allow comparison of results between laboratories and be able to define viral load thresholds in clinical guidelines it is of importance that laboratory assay results generate comparable viral load values with known accuracy and precision. The recently introduced International Standard for CMV in combination with regular external quality assessment of CMV molecular diagnostics may play an important role in the interlaboratory comparison of CMV viral load results. Molecular methods are also applied for the detection and diagnosis of CMV infection in newborn infants. The diagnosis of a congenital CMV infection is frequently based on the detection of CMV DNA in dried blood spots (DBS) of the newborn collected early after birth. Similar to the situation for viral load assays, DBS based assays for CMV DNA detection are heterogeneous with regard to their setup, performance

and sensitivity. Analytical aspects of assay design will be discussed in relation to the clinical sensitivity requirements and in relation to results of external quality assessments for detection of CM in DBS samples from newborns.

350 - Session B.04: Virus and Host

Date: 02/05/2017

Time: 14:15 - 15:45 hrs

Invited presentation

B-INV-004

Impact of placental immune responses on congenital HCMV transmission and pathogenesis

Dana Wolf

Hadassah University Hospital, Clinical Virology; Clinical Microbiology & Infectious Diseases, JERUSALEM, Israel

In view of the severe outcome associated with congenital HCMV infection, there is an urging need to better understand the innate mechanisms acting to limit trans-placental viral transmission. The placenta, given its critical function in protecting the developing fetus, has evolved effective, yet largely uncharacterized, innate immune barriers against invading pathogens. Recent studies, further facilitated by the Zika virus (ZIKV) epidemic, have begun to uncover the importance of these local-placental innate immune responses in the defense against congenital infections. To gain a global insight into these earliest events of viral-tissue interplay within the authentic environment of the maternal-fetal interface, we have employed ex vivo HCMV infection and genome-wide transcriptome analysis in maternal-decidual (the maternal aspect of the placenta) and chorionic villi (the fetal aspect of the placenta) tissues, maintained as integral 3D organ cultures. We further compared the innate tissue-response patterns following parallel infections with HCMV and ZIKV, and explored their impact on viral transmission and damage in the maternal-fetal interface. These studies in authentic human tissues provide a novel insight into the front-line placental innate responses which could mediate the outcome of congenital infection.

350 - Session B.04: Virus and Host

Date: 02/05/2017

Time: 14:15 - 15:45 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-014

Lessons from an epidemic: Patterns of Cytomegalovirus and Zika virus infection modeled in the human placenta ex vivo

Lenore Pereira¹, Takako Tabata¹, Matthew Pettitt¹, Henry Puerta-Guardo², Daniela Michlmayr², Eva Harris²

¹University of California San Francisco, Cell and Tissue Biology, SAN FRANCISCO, CA, United States of America

²School of Public Health, University of California Berkeley, Division Infectious Disease & Vaccinology, BERKELEY, CA, United States of America

Human cytomegalovirus (HCMV), a member of the *Herpesvirus* family, is a leading viral cause of congenital infection and permanent birth defects – microcephaly, neuromotor deficits, impaired hearing and vision loss. Zika virus (ZIKV), a member of the *Flavivirus* family, is responsible for the recent pandemic in the Americas, and like HCMV, causes devastating birth defects. Intrauterine growth restriction, a placental defect, is often present in cases of congenital infection and women infected for the first time during pregnancy have prolonged viremia and an increased risk of fetal infection. How these viruses disseminate to the placenta and undermine development is poorly understood. In numerous studies, we characterized the molecular mechanisms of HCMV infection that undermine

differentiation of placental cells leading to pathogenesis. Recently, we reported ZIKV infects diverse primary cell types from human placentas and amniochorionic membranes, and replicates in first-trimester explants of chorionic villi producing infectious progeny (Tabata, *et al.* Cell Host & Microbe, 2016). For both viruses, we observed reproducible patterns of infection in cytotrophoblasts (CTBs) in columns of proliferating cells and found strain-specific variation in apoptosis and invasion of the extracellular matrix. In contrast to clearance of immune complexes of IgG and HCMV virions by Hofbauer cells, ZIKV replicated in these cells, located near newly developing cell columns and branching villi. Both viruses replicated poorly in amniotic epithelial cells from late-gestation placentas. Inflammatory cytokines secreted into conditioned medium, associated with innate immune responses, contributed to reduced titers in infected chorionic villi and the amniotic epithelium. Our studies suggest HCMV and ZIKV disseminate in the maternal and fetal compartments and that host factors initiate innate immune responses with the potential to limit infection during gestation.

350 - Session B.04: Virus and Host

Date: 02/05/2017

Time: 14:15 - 15:45 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-015

A clinical CMV isolate passaged on fibroblasts in the presence of hyperimmunoglobulin retains epithelial tropism and an intact UL128-131A locus

Michael McVoy¹, Xiaohong Cui¹, Dirk Dittmer², Mark Schleiss³

¹Virginia Commonwealth University, Department of Pediatrics, RICHMOND, United States of America

²University of North Carolina at Chapel Hill, CHAPEL HILL, United States of America

³Center for Infectious Diseases and Microbiology Translational Research, MINNEAPOLIS, United States of America

Propagation of human cytomegalovirus (CMV) in cultured cells results in genetic alterations that are associated with improved growth *in vitro* and significant attenuation *in vivo*. For example, mutations in the *UL 128-131A* locus consistently emerge upon fibroblast passage. These mutations disrupt expression of proteins forming a pentameric complex that is necessary for infection of epithelial or endothelial cells but is dispensable for replication on fibroblasts. That such mutations arise suggests that expression of a functional pentameric complex has deleterious effects on CMV replication in fibroblasts. Conversely, that similar mutations have not been observed for CMV in clinical material suggests that selection against the pentameric complex does not manifest *in vivo*. Given that replication of CMV *in vivo* generally occurs in the context of CMV-specific antibodies, we reasoned that cell culture virus propagation would more accurately model replication *in vivo* if CMV-specific antibodies were present in the culture medium, and consequently, the accumulation of certain adaptive mutations might be mitigated. To test this hypothesis, CMV in urine from a congenitally infected newborn was used to infect replicate fibroblast cultures. One lineage was passaged with hyperimmunoglobulin (HIG) in the culture medium while the other was passaged in the absence of HIG. Epithelial tropism was lost by passage nine in the absence of HIG, while virus passed 20 times with HIG retained epithelial tropism. Genome sequencing confirmed that the HIG-passaged stock retained an intact *UL 128-131A* locus at passage 22, while a frame-shift mutation disrupting *UL 131A* was observed in the stock passaged without HIG. As disruption of the pentameric complex has been proposed to favor release of cell-free virus from infected fibroblasts, it is probable that antibody neutralization of cell-free virus forces virus amplification to occur exclusively by cell-to-cell spread, thereby alleviating the selective pressure favoring disruption of the *UL 128-131A* locus. Analogous effects likely manifest *in vivo*.

350 - Session B.04: Virus and Host

Date: 02/05/2017

Time: 14:15 - 15:45 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-016

CRISPR/Cas9-mediated genome editing of human cytomegalovirus limits productive infection

Emmanuel Wiertz, Ferdy Van Diemen, Elisabeth Kruse, Saskia Imhof, Robert Jan Lebbink
University Medical Center Utrecht, Department of Medical Microbiology, UTRECHT, Netherlands

Human cytomegalovirus (HCMV) is the most common viral cause of congenital defects and is responsible for serious disease in immuno-compromised individuals. HCMV persist in its host for life by establishing a latent infection that is interrupted by periodic reactivation events during which replication occurs. Current antiviral drug treatments mainly aim at targeting the clinical manifestations of the productive stage, but they are ineffective at eliminating the virus from the infected host. We set out to combat HCMV infection by exploiting the CRISPR/Cas9 system to target viral genetic elements important for viral fitness. By targeting gRNAs to essential viral genes, we show effective abrogation of HCMV replication. Expression of gRNAs targeting nonessential HCMV genes induced mutations within these genes, but this did not interfere with HCMV replication. Apparently, effective interference with HCMV replication requires modification of essential genes. Our studies indicate that the CRISPR/Cas9 system can be effectively targeted to HCMV genomes as a potent anti-viral strategy impairing viral replication.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-002

The role of anti-Cytomegalovirus CD8 T cell responses in adipose metabolic dysregulation and diabetes.

Nico Contreras, Megan J. Smithey, Janko Nikolich-Zugich
The University of Arizona, Immunobiology, TUCSON, United States of America

Cytomegalovirus (CMV) infection results in a lifelong and persistent infection that is characterized by stochastic bouts of replication. Furthermore, the primary and definitive location of viral replicative senescence has yet to be identified. As CMV has a broad tissue and cellular tropism the identification of a 'viral reservoir' has been difficult. The objective of this study was to investigate the potential involvement of adipose tissue in acute and chronic immune responses during CMV infection. Adipose tissue is a highly heterogeneous tissue containing the adipocytes and stromal vascular fraction (SVF). The SVF consists of numerous immune cells and specifically CD8a T cells, which are crucially important for the control of CMV infection. Inflammation within adipose tissue has been increasingly investigated in the context of obesity, but whether or not CMV infects adipose tissue and the resulting downstream consequences of such an infection has not been reported. Here we demonstrate, using the mouse model of CMV infection (mCMV) that mCMV is capable of infecting the cellular constituents of adipose tissue and this results in a significant CD8a+ T cell response that is maintained in both the acute and lifelong infection, possibly leading to a decline in metabolic function. These results have far reaching implications for metabolic health, increase our knowledge of mCMV tropism, and identify a neglected reservoir for viral replication and persistence.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-005

Excessive Reactive Oxygen Species and Murine CMV Labyrinthitis: A Possible Target for Therapy?

Albert Park¹, Phayvanh Sjogren¹, Ali Almishaal¹, Pranav Mathur², Taelor Johnson¹, Matt Firpo¹

¹University of Utah, SALT LAKE CITY, United States of America

²Otonomy Inc, SAN DIEGO, United States of America

INTRODUCTION: Cytomegalovirus (CMV) infection is the leading nonhereditary cause of pediatric sensorineural hearing loss (SNHL). Treatment with antiviral therapy has potentially devastating side effects. Antioxidant therapy may offer a novel and safe treatment for CMV-induced SNHL.

OBJECTIVES: The goals of the study were to 1) determine whether reactive oxygen species (ROS) mediates CMV-induced SNHL and 2) evaluate the protective effects of the antioxidants D-methionine and vitamins A, C, E and magnesium (ACE-Mg) in a murine model.

METHODS: Nrf2 knockout mice were inoculated with murine cytomegalovirus (mCMV) on postnatal day 3 (P3). Auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) were performed on these and uninoculated controls. Next, mCMV-infected BALB/c mice were treated with D-methionine and ACE-Mg for 14 days. Temporal bones were harvested at P10 and stained with dihydroethidium (DHE), a marker of ROS. Scanning electron microscopy (SEM) to evaluate outer hair cell (OHC) integrity and ABR/DPOAE were performed at P30.

RESULTS: Nrf2 mCMV-infected mice had worse hearing (ABR) than uninfected Nrf2 mice ($P < 0.001$). DHE was increased in BALB/c infected mice compared to uninfected mice. Compared to untreated infected controls, D-methionine and ACE-Mg treated mice demonstrated an attenuation of DHE fluorescence, improvement in ABR and DPOAE thresholds relative to untreated infected controls ($P < 0.0001$), and decreased OHC loss by SEM.

CONCLUSION: Excessive ROS mediates CMV-induced hearing loss in this murine model. The improved OHC and hearing outcomes in mCMV-infected mice treated with D-methionine or ACE-Mg suggest potential therapeutic utility and rationale for a human trial.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-007

Interferon-induced transmembrane protein 3 prevents cytokine-driven cytomegalovirus pathogenesis

Ian Humphreys¹, Maria Stacey¹, Simon Clare², Clement Mathew¹, Simon Jones¹, Paul Kellam²

¹Cardiff University, Heath Park, CARDIFF, United Kingdom

²Wellcome Trust Sanger Institute, HINXTON, United Kingdom

The antiviral restriction factor Interferon-induced transmembrane protein 3 (IFITM3) inhibits cell entry of a number of viruses and genetic diversity within *IFITM3* determines susceptibility to viral disease in humans. Using the murine cytomegalovirus (MCMV) model of infection, we discovered that *Ifitm3* limits herpesvirus-associated pathogenesis without directly preventing virus replication. Instead, *Ifitm3* promoted antiviral cellular immunity through the restriction of virus-induced lymphopenia, apoptosis-independent NK cell death and loss of T cells. Viral disease in *Ifitm3*^{-/-} mice was accompanied by elevated production of cytokines, most notably interleukin-6 (IL-6). *Ifitm3* inhibited IL-6 production by myeloid cells in response to replicating and non-replicating virus, and also following stimulation with TLR ligands Poly(I:C) and CpG. Although IL-6 promoted virus-specific T cell responses, uncontrolled IL-6 expression in *Ifitm3*^{-/-} mice triggered the loss of NK cells, and subsequently impaired control of MCMV replication. Thus, *Ifitm3* represents a checkpoint regulator of anti-viral immunity, which controls cytokine production to restrict viral pathogenesis. These data suggest the utility of cytokine-targeting strategies in the treatment of virus-infected individuals with impaired IFITM3 activity.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-010

High rhesus cytomegalovirus shedding frequency is associated with Th2-biased CD4 and attenuated CD8 T-cell responses following subcutaneous challenge

William Chang, Jaewon Lee, Jesse Deere, Luis Castillo, Hung Kieu, Yujuan Yue, Kimberli Schmidt, Sungjin Kim, Peter Barry

Center for Comparative Medicine, University of California, Davis, DAVIS, United States of America

Prolonged shedding of virus in saliva following primary cytomegalovirus (CMV) infection is considered as the major source of horizontal transmission to susceptible hosts. The dynamics of viral shedding are likely governed by properties of CMV infectivity and host immune responses. In this study, we investigated the factors contributing to the variation of shedding frequency in saliva following primary rhesus CMV (RhCMV) infection using the nonhuman primate model. A total of 46 naïve juvenile rhesus macaques (1-4 years old) were challenged with RhCMV_{UCD59} by subcutaneous inoculation (SC; $n=15$) or through oral exposure (PO; $n=31$). We assessed shedding of RhCMV via real-time PCR using saliva collected with oral swabs and antiviral humoral responses by IgG ELISA for a window of 10 weeks post challenge (from week 6 to week 16). The results reveal that higher oral shedding frequencies are (1) significantly associated with the route of RhCMV challenge ($P=0.0022$) and (2) highly correlated with robust antibody responses ($P=0.0012$). To better understand the immunological factors associated with the dynamics of RhCMV shedding, comprehensive analyses were conducted using samples collected from two groups of naïve animals challenged with RhCMV SC ($n=8$) and PO ($n=9$). Lower oral shedding frequencies, in conjunction with slightly delayed but prolonged RhCMV-specific CD8 T-cell responses, were observed in PO challenged monkeys. In contrast, animals challenged SC shed virus at higher frequencies and exhibited enhanced RhCMV-specific CD4 T-cell responses. The presence of these Th2-biased CD4 T-cells (producing IL-4 and IL-13 after RhCMV antigen stimulation) resulted in increased antiviral IgG titers and extended elevation of activated memory B-cells in circulation. These data demonstrate distinct outcomes of virus shedding patterns and host antiviral immune responses induced by different routes of virus challenge, which may be caused by initial interplay of CMV and host innate immunity at the site of infection.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-014

Conserved death-receptor signaling inhibition identified by a chimeric mouse CMV expressing a homologous human CMV gene

M. Zeeshan Chaudhry¹, Bahram Kasmajour¹, Carlos Plaza¹, Milica Bajagic¹, Rosaely Casalegno-Garduño¹, Lisa Borkner¹, Tihana Lenac², Andrea Scrima¹, Ingo Schmitz¹, Luka Cicin-Sain¹

¹Helmholtz Center for Infection studies, Vaccinology, BRAUNSCHWEIG, Germany

²Faculty of Medicine, University of Rijeka, Center for Proteomics, RIJEKA, Croatia

Apoptosis is an important defense mechanism mounted by the immune system to control virus replication. Hence, cytomegaloviruses (CMV) evolved and acquired numerous anti-apoptotic genes. vICA, the product of the human CMV (HCMV) UL36 gene, binds to pro-caspase-8, thus inhibiting death-receptor apoptosis and enabling viral replication in differentiated THP-1 cells. *In vivo* studies of the function of HCMV genes are severely limited by the strict host specificity of cytomegaloviruses, but CMV orthologues that co-evolved with other species allow the experimental study of CMV biology *in vivo*. The mouse CMV (MCMV) homologue of the UL36 gene is called M36, and its protein product (pM36) is a functional homologue of vICA that binds to murine caspase-8 and inhibits death-receptor mediated apoptosis. M36-deficient MCMV is severely growth impaired in macrophages and *in vivo*. Here we show

a chimeric MCMV expressing the UL36 ORF sequence instead of the M36 one. The newly generated MCMV^{UL36} was tested for growth and apoptosis in macrophage lines RAW 264.7, J774A.1 and IC-21. Apoptosis occurred upon infection with the MCMV lacking M36, but not in macrophages infected with wild-type MCMV or MCMV^{UL36}. Likewise, virus growth was rescued by UL36 expression, both in macrophages and *in vivo* in the liver and spleen of BALB/c and C57BL/6 mice. However, the virus replication was only partially rescued in the salivary glands of both mouse strains. Finally, we showed that pUL36 binds to the murine pro-caspase-8. In conclusion, this is to the best of our knowledge the first report of an immune-evasive HCMV gene that is conserved enough to functionally replace its MCMV counterpart and thus allow its study in an *in vivo* setting. As UL36 and M36 proteins engage the same molecular target on the host side, our newly developed model can facilitate studies of anti-viral compounds against the UL36 gene *in vivo*.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-025

Pre-infection infusion of hyperimmune globulin (HIG) can protect rhesus macaques against congenital CMV transmission

Amitinder Kaur¹, Dollnovan Tran¹, Kristy Bialas², Michael Cohen-Wolkowicz², Huali Wu², Meng Chen², Nathan Vandergrift³, Margaret Gilbert¹, Cody Nelson², Peter Barry⁴, Sallie Permar²

¹Tulane National Primate Research Center, COVINGTON, United States of America

²Duke Human Vaccine Institute, DURHAM, United States of America

³Duke Human Vaccine Institute, DURHAM, United States of America

⁴California National Primate Research Center, DAVIS, United States of America

Development of an effective maternal vaccine offers the best hope for protection against congenital CMV but is hampered by an incomplete understanding of the viral and immune determinants of intrauterine transmission. We recently reported on a novel rhesus macaque model of congenital CMV infection and showed that rhesus CMV (RhCMV) inoculation of CD4+ T cell-depleted, RhCMV-seronegative dams early in the second trimester resulted in placental transmission in all dams and spontaneous fetal abortion in 5 of 6 infected dams. To evaluate the protective role of antibodies in this model, we tested the effect of passive infusion of HIG purified from the plasma of naturally-infected RhCMV-seropositive macaques with high titers of binding RhCMV-specific IgG antibodies. A single intravenous dose (100mg/kg) of "standard" preparation HIG one hour prior to RhCMV inoculation protected all 3 dams against fetal loss but prevented placental transmission in only 1 of 3 (33%) dams. Two infusions of a dose-optimized "high-potency" HIG preparation with 4-fold higher anti-RhCMV epithelial cell neutralization titers administered one hour prior to and three days after RhCMV inoculation resulted in sustained titers of RhCMV IgG for 10-14 days, lowered peak plasma RhCMV levels, and protected all (3/3) dams against placental transmission and fetal loss. Overall, the HIG-infused group showed a significant reduction in placental transmission and increased fetal survival compared to the control macaques ($P < 0.05$; Heinze-Macro exact log-rank test). More than 300 genes were differentially-expressed in the placenta of RhCMV-transmitting and non-transmitting dams, and RhCMV transmission was associated with upregulation of innate immune pathway genes including several specific for natural killer cell localization/function. Our data suggest that pre-existing, potentially-neutralizing IgG can protect against congenital CMV transmission and disease. These studies further validate the utility of the rhesus macaque model in evaluating immune determinants of protection against congenital CMV infection.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-029

Insights into the pathogenesis of wild-type rhesus cytomegalovirus strains upon multiple oral mucosa inoculations

Yujuan Yue¹, William Chang¹, Julia Li², Kimberly Schmidt¹, Nancy Nguyen¹, Andrew Lehman¹, Xinzhen Yang², Peter Barry¹

¹University of California, Davis, DAVIS, United States of America

²Vaccine R&D, Pfizer Inc, PEARL RIVER, NY10965, United States of America

Rhesus cytomegalovirus (RhCMV) infection of macaques is a clinically relevant nonhuman primate model to study human CMV (HCMV) persistence and pathogenesis. Most *in vivo* RhCMV experiments have utilized the fibroblast-adapted 68-1 strain via either intravenous or subcutaneous routes of inoculation. However, given the genetic and biological changes associated with 68-1, investigations of the pathogenesis of wild-type RhCMV strains delivered by a natural transmission route, such as the oral mucosa, would be more clinically relevant for rigorously evaluating HCMV vaccine strategies. In this study, by using a wild-type RhCMV strain, UCD52 and a related strain, UCD59, we demonstrated that systemic infection and frequent shedding were established by multiple oral mucosa inoculations. Further analysis of the tissues of UCD52-infected monkeys showed that RhCMV disseminated to a broad range of tissues including brain, spinal cord and reproductive organs; however, the most commonly infected organs were thymus, spleen, lymph nodes, kidneys, bladder and salivary glands. Gross pathology and histology examination found multiple nodules in spleen and variable levels of lymphofollicular hyperplasia in both spleen and lymph nodes. In addition, limited virus dissemination was unexpectedly observed in one monkey that exhibited extremely weak RhCMV antibody responses. Together, we have established an oral RhCMV infection model that mimics natural HCMV infection in horizontal transmission and have characterized virological and immunological parameters that will be useful for future application of this model in HCMV vaccine evaluation.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-052

Neonatal CMV infection-induced learning disabilities and impaired neurocognitive performance in the Morris Water Maze associated with CD4+/CD8+ neuroinflammatory infiltrates

Mark Schleiss¹, Claudia Fernández-Alarcón¹, Lucy Meyer¹, James Lokensgard¹, Michael Benneyworth², Nelmary Hernandez-Alvarado¹, Bradley Janus¹, Michael McVoy³

¹Centre for Infectious Diseases and Microbiology Translational Research, University of Minnesota Medical School, Department of Pediatrics, MINNEAPOLIS, United States of America

²University of Minnesota Medical School, Department of Neurosciences, MINNEAPOLIS, United States of America

³Virginia Commonwealth University Medical School, Department of Pediatrics, RICHMOND, VIRGINIA, United States of America

Congenital cytomegalovirus (cCMV) is a leading infectious cause of neurologic deficits in children. The purpose of this study was to explore the impact of systemic neonatal guinea pig cytomegalovirus (GPCMV) infection on brain histology, inflammation and neurocognitive function in guinea pigs using a Morris water maze (MWM) model coupled with histopathological analyses. Eight pups were inoculated (intraperitoneal route) with a virulent, recombinant TurboFP635 red fluorescent protein (RFP)-expressing GPCMV within 96 hours of birth (5×10^6 PFU). Six pups were mock-infected. On days 15-19 post-infection (pi), the animals were subjected to testing in the MWM to evaluate learning and memory. Viral load in blood and tissue was determined by qPCR. Brain samples were collected to examine for histological abnormalities. Viremia was detected at day 3 pi in 7/8 experimentally infected animals. End-organ dissemination occurred in brain, lung, liver, and spleen. Experimentally infected animals demonstrated brain damage evidenced by neuronal necrosis and dilated lateral ventricles. Double-stained sections with CD4+ and CD8+ T-cell and GFAP markers revealed that areas of increased inflammatory cells were associated with a prominent reactive astrogliosis. CD8+ T cells predominated, and some cell clusters were surrounded by intensely stained GFAP-positive astrocytes. Iba1⁺ stained

cells with highly branched processes, suggestive of activated microglia, were observed in GPCMV-infected pup brains. Significant cognitive differences were observed between infected and uninfected pups, as assessed by measurement of total distance traveled and escape latencies in the MWM ($p < 0.0001$). Additionally, the infected animals crossed the target platform zone significantly fewer times/testing interval (0.89 crosses) than controls (2.28; $p < 0.05$). In summary, neonatal infection in guinea pigs produced brain damage, leading to significant cognitive and learning defects, as evaluated by histology and the MWM. This model should prove valuable in evaluation of interventions targeting the prevention of neuropathogenic and neurodevelopmental sequelae caused by neonatal CMV infections.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-053

Cytomegalovirus (CMV) Transmission by Breast Milk: Establishment of a Small Animal Model

Elizabeth Swanson, Craig Bierle, Claudia Fernández-Alarcón, Jason Zabeli, Mark Schleiss
University of Minnesota, MINNEAPOLIS, United States of America

Background: CMV reactivates in nearly all seropositive mothers during lactation, and transmission through milk may lead to severe symptoms in premature infants. An animal model of milk transmission would allow for interrogation of immune correlates of protection and vaccine effects on lactation-associated reactivation and transmission. This study characterizes the viral kinetics of guinea pig CMV shedding in milk.

Methods: Two groups of pregnant outbred female Hartley guinea pig dams were challenged with 1.5×10^6 PFU guinea pig CMV (GPCMV) on the day of delivery, or sham-infected. Group 1 (N=5) animals were GPCMV seropositive, and Group 2 (N=6) animals were seronegative prior to the experiment. Blood and milk samples were collected on days 0, 7, and 14 following delivery for viral load quantification by qPCR.

Results: Group 1 dams showed little to no CMV in blood and milk. 1 of 3 challenged dams had low viral loads by day 7 in blood (9.2×10^2 GPCMV genome copies/mL) and milk (3.4×10^2 copies/mL). 1 of the 2 sham-infected seropositive dams had only early transient viremia (2.0×10^2 copies/mL on day 0). In contrast, all Group 2 dams challenged with GPCMV (n=4) had larger and more sustained viremia and virolactia. Mean peak viral load at day 7 was 2.4×10^4 copies/mL in blood (range $8.0 \times 10^3 - 5.2 \times 10^4$ copies/mL, SD $\pm 2.0 \times 10^4$), and 6.0×10^5 copies/mL in milk (range $7.6 \times 10^3 - 1.7 \times 10^6$ copies/mL, SD $\pm 8.1 \times 10^5$).

Discussion: This study demonstrates that in guinea pigs, as in humans, GPCMV is shed in milk during lactation. Viral loads were similar to that in blood, peaking at day 7 following delivery in previously naïve guinea pigs. Pre-existing humoral immunity to GPCMV was protective against viremia and viral shedding in milk following re-infection. Future directions will include evaluating pup tissues for evidence of milk transmission, and humoral and cellular immune responses associated with protection.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-057

UL128 Homolog Mutants Form Pentameric Complexes but Recombinant Viruses have Attenuated Cell Tropism and Pathogenicity in the Guinea Pig Model

Alistair McGregor, Stewart Coleman, K. Yeon Choi
Texas A&M University Health Science Center, Microbial Pathogenesis & Immunology, COLLEGE STATION, United States of America

Guinea pig CMV (GPCMV) encodes a homolog pentameric (PC) complex (gH, gL, GP129, GP131, GP133) for virus cell tropism (eg. epithelial cells) and efficient congenital infection. The PC is an important neutralizing target antigen. An improved understanding of the GPCMV PC is necessary for development of pre-clinical CMV intervention strategies. Deletion of the C-terminus (77 codons) of a UL128 homolog (GP129) resulted in: lack of PC formation; loss of viral epithelial tropism; impaired pathogenicity; decreased congenital infection. To investigate the function of the GP129 C-terminus domain, three mutants were constructed deleting codons: 102- 120 (DEL1); 121-140 (DEL2); or 145-178 (DEL3). These mutants systematically deleted hydrophilic conserved regions found in HCMV UL128. Transient expression demonstrated that all mutants formed a PC unlike GP131 and GP133 C-terminal mutants. Recombinant GPCMV encoding mutant GP129s (DEL1-3) resulted in impaired tropism for renal and newly isolated placental trophoblast epithelial cells. GPCMV DEL1 and DEL2 mutants were highly impaired, whereas a DEL3 mutant had a 2 log reduction in growth. Fibroblast growth was not affected. Additionally, UL128 has a potential monomeric chemokine function. In a series of studies, the significance of the presumptive CC chemokine motif encoded by GP129 was investigated. The CC (codons 26 -27) and cysteines (codons 47 and 62) were separately altered by alanine substitution. The GP129 chemokine mutants were capable of forming PC in transient studies. However, GPCMV encoding GP129 chemokine mutants resulted in a 2-3 log reduction for viral growth on epithelial cells. Pathogenicity studies with one GP129 chemokine mutant virus had impaired yields in target tissue and failed to be detected in liver or salivary glands between 4-27dpi. Curiously, it exhibited extended viremia throughout the study unlike wild type virus. Overall, correct PC formation is dependent upon critical GP129 sequences which require evaluation by recombinant virus tropism and pathogenicity studies.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-058

Congenital CMV infection is associated with Sensorineural Hearing Loss (SNHL)

William Britt¹, Cathy Yea Won Sung¹, Keiko Hirose², Stipan Jonjic³, William Britt¹

¹University of Alabama at Birmingham, Pediatrics, BIRMINGHAM, United States of America

²Washington University, Otolaryngology, ST. LOUIS, United States of America

³University of Rijeka, Embryology and Histology, RIJEKA, Croatia

Background

An estimated 10% of infants with congenital CMV develop hearing loss. Mechanisms of hearing loss following CMV infection in utero remain undefined. We developed a murine model of hearing loss in congenital CMV infection using IP inoculation newborn mice that results in hematogenous spread to the inner ear. Hearing loss occurred in up to 60% of infected mice and was dependent on the size of the viral inoculum. We have used this model to investigate the impact of cochlear infection on auditory pathway development.

Methods

Mouse cytomegalovirus (MCMV) was injected IP in P1 newborn Balb/c mice. Cochleas from infected mice were assayed utilizing different techniques for detection of virus, inflammatory cells, and cochlea structures on PNd 8-78. Hearing (ABR) was measured in control and infected mice.

Results

Threshold shift of 15-20 dB across all frequencies was found in mice with hearing loss after MCMV infection. Virus was detected in the cochlea as early as 4 days post infection in the lateral wall and in the ganglion and persisted for over 6 months. Inflammatory cells were abundant in the inner ear at P12 and included lymphocytes, natural killer cells, and monocytes, but not neutrophils. The hair cells and organ of Corti were uniformly preserved after MCMV infection, even in mice with hearing loss; however, spiral ganglion neuron numbers were decreased in mice with hearing loss. Numerous genes that initiate inflammation and cell death pathways were activated in the infected cochlea.

Conclusions

Following hematogenous spread, MCMV infected cells in the lateral wall and spiral ganglion. Threshold shifts were observed in a significant percentage of mice. Hearing loss was associated with spiral ganglion neuron loss but not with hair cell loss. Ongoing studies are aimed at identifying specific

mechanism of hearing loss in this model.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-008

The antiviral cGAS-STING-mediated type I interferon response to murine cytomegalovirus (MCMV) is inhibited by the multifaceted MCMV m152 membrane protein

Melanie Brinkmann¹, Markus Stempel¹, Baca Chan¹, Vanda Juranic Lisnic², Stipan Jonjic²

¹Helmholtz Centre for Infection Research, BRAUNSCHWEIG, Germany

²Department of Histology and Embryology/ Center for Proteomics Faculty of Medicin, RIJEKA, Croatia

The type I interferon (IFN) response mediated by pattern recognition receptors (PRR) is imperative for the establishment of the early antiviral immune response. In macrophages, the herpesvirus murine cytomegalovirus (MCMV) is sensed by the PRR cyclic GMP-AMP synthase (cGAS), which senses DNA in the cytoplasm. The cGAS-produced second messenger cGAMP subsequently activates the endoplasmic reticulum (ER)-resident adaptor protein stimulator of interferon genes (STING), which induces type I IFN transcription.

To identify MCMV proteins that negatively modulate the cGAS-STING-induced type I IFN response, we screened an MCMV cDNA library in an unbiased luciferase-based reporter assay and identified the ER-resident m152 membrane protein as a novel negative regulator of the STING-dependent type I IFN response.

We show that the transcriptional activation and secretion of IFN β downstream of the cGAS-STING pathway is selectively inhibited in macrophages stably expressing m152. In line with this result, an MCMV mutant lacking m152 induces elevated type I IFN responses compared to wildtype MCMV *in vitro* as well as *in vivo*. Under resting conditions, STING is localized in the ER. Upon stimulation and infection, it rapidly translocates from the ER to the Golgi apparatus. We find that in resting cells, m152 and STING colocalize and interact in the ER. Upon stimulation, both proteins traffick to the Golgi and still interact. Interestingly, in the absence of STING, m152 does not traffick to the Golgi upon stimulation. Notably, in the presence of m152, the translocation and subsequent degradation needed for active signaling of STING is delayed. Taken together, we have identified m152 as the first MCMV protein that engages the key adaptor protein STING to inhibit type I IFN signaling. With this study, we have enriched the immune evasion repertoire of m152, which targets multiple arms of the immune response by antagonizing innate signaling, Natural Killer- and T-cell dependent responses.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-016

CD4 and CD8 T cell responses to human cytomegalovirus proteins expressed by latently infected cells

George Sedikides, Sarah Jackson, Georgina Brown, John Sinclair, Mark Wills

University of Cambridge, Medicine, CAMBRIDGE, United Kingdom

During HCMV latency, the viral transcription programme is very limited compared to lytic infection. We have previously shown that two viral gene products expressed during latency, UL138 and LUNA, are recognised predominantly by CD4+ T cells and a subpopulation of these cells secrete the immunosuppressive cytokines IL-10 and transforming growth factor beta (TGF- β).

Little is known about the host immune response to other latency-associated viral proteins; US28,

UL111a, and UL144. By dual IFN γ /IL-10 FluoroSpot assay, we now show that these viral gene products are also recognized by CD4⁺ T cells and these CD4⁺ T cell responses are composed of distinct T cell populations that secreted either IFN γ or IL-10. The high sensitivity of this assay has also revealed previously uncharacterised CD8⁺ T cell responses to US28, UL111a, and UL144, as well as responses to LUNA and UL138. Intriguingly, we have also observed IL-10 secretion by a distinct population of latency-specific CD8⁺ T cells.

We have also analysed these latency-associated antigen specific CD4⁺ and CD8⁺ T cells for CD107a expression as an indicator of cytotoxic potential. Our results show that CD107a is expressed by a sub-population of both CD4⁺ and CD8⁺ T cells, although of note, significantly fewer UL111a and UL138 specific CD8⁺ T cells expressed CD107a compared to LUNA, US28 and UL144.

Most individuals make a Th1 anti-viral CD4⁺ and/or CD8⁺ CTL response to at least one latency-associated protein, and some of these T cells clearly express cytotoxic capacity, yet clearly, these responses are unable to clear latently infected cells as latency persists for life. We hypothesise that the immunosuppressive IL-10 and TGF- β present in the microenvironment could be restricting anti-viral T cell functions against latently infected cells. Current work focuses on neutralising these inhibitory cytokines to determine if this would then lead to the clearance of latently infected cells.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-021

The role of HLA-C, HLA-E and HLA-G in the long-term outcome of congenital Cytomegalovirus infection

Roberta Rovito, Frans Claas, Geert Haasnoot, Dave Roelen, Michael Eikmans, Marjolein Korndewal, Louis Kroes, Ann Vossen

Leiden University Medical Center (LUMC), Medical Microbiology, LEIDEN, Netherlands

Congenital Cytomegalovirus infection (cCMV) causes long-term impairments (LTI) in half of children symptomatic at birth and in 13.5% of asymptomatic ones. This study evaluates if unique maternal-fetal HLA combinations influence cCMV outcome.

96 children with cCMV and their mothers were typed for HLA-C-E-G. A commercial Luminex technique for HLA-C and two TaqMan assays for HLA-E-G polymorphisms, associated with protein levels, were used. Mismatches and missing-self were calculated based on the number of alleles present in the child but not in mother and viceversa. Statistical significance was assessed by using a Chi-squared test.

The results showed a higher % of symptomatic neonates with HLA-C2 and HLA-E mismatches than asymptomatic (31.6% vs 10.5% $p=0.021$, 52.6% vs 21.1% $p=0.006$). Additionally, higher % of mothers of children with LTI had HLA-C missing-self than mothers of children without (96.2 vs 65.2, $p=0.002$). Finally, a higher % of mothers of children with LTI was homozygous for HLA-G deletion (i.e. higher proteins levels) than mothers of children without (55.6% vs 26.1% $p=0.023$). Neonates of HLA-G del/del mothers had trend significant higher viral loads ($p=0.06$).

Based on these findings, we hypothesised that higher maternal HLA-G levels may lead to a favourable environment for CMV, facilitating replication, dissemination and delaying maternal response. This reduced viral control may induce increased placental viral loads and direct damage. Fetal-maternal HLA mismatches have shown to increase lymphocyte activation without pregnancy complications. If the increased placental lymphocytic influx and decreased activation threshold, caused by cCMV, occur at the backdrop of HLA mismatches, an indirect placental damage could occur. Finally, the post-natal long-term inefficient CMV control might be partially due to non-inherited maternal antigens (NIMA) exposition, which induces an active mechanism of immune-suppression. This study gives useful insights into cCMV pathogenesis in all compartments involved and could help to identify neonates with a worse outcome.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-048

Polymorphisms in human cytomegalovirus gO may confer escape from neutralization by antibodies targeting epitopes in gH/gL

Michael McVoy¹, Xiaohong Cui¹, Daniel Freed², Dai Wang², Tong-Ming Fu², Ping Qiu², Fengsheng Li²
¹Virginia Commonwealth University, Department of Pediatrics, RICHMOND, United States of America
²Merck & Co., KENILWORTH, United States of America

Cytomegalovirus (CMV) entry into fibroblasts requires the trimeric gH/gL/gO complex while entry into epithelial cells requires an additional pentameric complex comprised of gH/gL/UL128/UL130/UL131A. Consequently, antibodies to epitopes within gH/gL have the potential to neutralize CMV entry into both cell types. However, certain neutralizing epitopes within gH/gL are strain-specific. To investigate this further, nine monoclonal antibodies targeting gH/gL were evaluated for fibroblast and epithelial neutralizing activities against epithelial tropic variants derived from CMV strains AD169, UxC, TB40/E, Towne, or NR. Four antibodies reacted with linear epitopes within residues 27-48 of gH from AD169 that are fully conserved in UxC and TB40/E. They did not bind to equivalent gH sequences from Towne, which contain four polymorphisms, and consistent with binding, these antibodies neutralized epithelial entry of AD169, UxC, and TB40/E, but not Towne. Surprisingly, these antibodies neutralized fibroblast entry of TB40/E but not AD169 or UxC. Five additional antibodies recognized conformational gH/gL epitopes and neutralized epithelial cell entry of all five strains and fibroblast entry of AD169, UxC, and TB40/E, but failed to neutralize fibroblast entry of strains Towne or NR. Thus, for both antibody groups neutralization mediated by antibody binding to gH/gL occurred during epithelial cell entry, while, for certain strains, the same antibodies failed to interact with the same gH/gL sequences to neutralize fibroblast entry. This suggests that antibody access to certain neutralizing epitopes in gH/gL can be restricted by polymorphisms that reside outside of gH/gL. That fibroblast neutralization is selectively impacted suggests that this restriction occurs in the context of gH/gL/gO rather than the pentameric complex, thus implicating gO as the likely restricting factor. If so, the polymorphic nature of gO may serve to promote viral escape from antibody neutralization during reinfection without directly altering functional gH/gL epitopes. These findings may have important implications for CMV vaccine or immunotherapeutic development.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-049

Human cytomegalovirus UL23 inhibits transcription of interferon- γ stimulated genes and blocks antiviral interferon- γ responses by interacting with N-myc interactor protein

Fenyong Liu¹, Linyuan Feng², Jingxue Sheng¹, Gia-Phong Vu¹, Yujun Liu³, Chingman Foo², Songbin Wu², Phong Trang¹, Marco Paliza-Carre¹, Yanhong Ran², Xiaoping Yang², Xu Sun², Zemin Deng⁴, Tianhong Zhou², Sangwei Lu¹, Hongjian Li²

¹University of California at Berkeley, BERKELEY, United States of America

²Jinan University, GUANGZHOU, China

³School of Medicine, ST. GEORGE'S UNIVERSITY, Grenada

⁴University of Southern California, LOS ANGELES, United States of America

Interferon- γ (IFN- γ) represents one of the most important innate immunity responses in a host to combat infections of many human viruses including human herpesviruses. Human N-myc interactor (Nmi) protein, which has been shown to interact with signal transducer and activator of transcription (STAT) proteins including STAT1, is important for the activation of IFN- γ induced STAT1-dependent transcription of many genes responsible for IFN- γ immune responses. However, no proteins encoded by herpesviruses have been reported to interact with Nmi and inhibit Nmi-mediated activation of IFN- γ immune responses to achieve immune evasion from IFN- γ responses. In this study, we provide the first direct evidence that UL23 protein of human cytomegalovirus (HCMV), a human herpesvirus, specifically

interacts with Nmi. This interaction was identified by the two-hybrid screen in yeast and co-immunoprecipitation in human cells. We showed that Nmi, when bound to UL23, was not associated with STAT1, suggesting that UL23 binding of Nmi disrupts the interaction of Nmi with STAT1. In cells overexpressing UL23, we observed (a) significant reduced levels of Nmi and STAT1 in the nuclei, the sites where these proteins act to induce transcription of IFN- γ stimulate genes, and (b) decreased levels of the induction of the transcription of IFN- γ stimulated genes. UL23-deficient HCMV mutants induced higher transcription of IFN- γ stimulated genes and exhibited lower titers than parental and rescued control viruses expressing functional UL23 in IFN- γ treated cells. Thus, UL23 appears to bind to Nmi and reduce the nuclear import and the availability of Nmi and its associated protein STAT1 for the activation of IFN- γ stimulated gene transcription, leading to a decrease of IFN- γ induced responses and an increase of viral resistance to IFN- γ . Our results further highlight the roles of UL23-Nmi interactions in facilitating viral immune escape from IFN- γ responses and enhancing viral resistance to IFN antiviral effects.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-001

Adaptive NKG2C⁺ NK cell response and the risk of cytomegalovirus infection in kidney transplant recipients

Miguel López-Botet¹, Dolores Redondo-Pachón², Marta Crespo², José Yélamos², Aura Muntasell², María J. Pérez-Sáez³, Silvia Pérez-Fernández², Joan Vila², Carlos Vilches⁴, Julio Pascual³

¹Hospital del Mar Medical Research Institute (IMIM). Univ. Pompeu Fabra, Immunology, BARCELONA, Spain

²Hospital del Mar Medical Research Institute (IMIM), Nephrology, BARCELONA, Spain

³Hospital del Mar, Nephrology, BARCELONA, Spain

⁴Instituto de Investigación Sanitaria Puerta de Hierro, Immunogenetics-Histocompatibility, MAJADAHONDA (MADRID), Spain

Cytomegalovirus (CMV) infection in kidney transplant recipients (KTR) has been associated with an increased risk of graft loss and reduced host survival. CMV promotes persistent expansions of NK cells expressing the CD94/NKG2C receptor. The *NKG2C* (*KLRC2*) gene is frequently deleted and copy number influences the adaptive response of NKG2C⁺ NK cells. The distribution of NKG2C⁺ NK cells and *NKG2C* genotypes (*NKG2C+/+*, *NKG2C+/del*, *NKG2Cdel/del*) were studied in cross-sectional (N=253) and prospective (N=122) KTR cohorts. Assessment of CMV viremia was restricted to symptomatic cases in the retrospective study, but was regularly monitored in the prospective cohort. Overall, the proportions of NKG2C⁺ NK cells were significantly higher in KTR who had suffered post-transplant symptomatic CMV infection in the cross-sectional study. Yet, along the prospective follow up (3, 6, 12 and 24 months), post-transplant NKG2C⁺ NK cell expansions were not observed in every patient with detectable viremia who received preemptive anti-viral therapy, suggesting that the adaptive NK cell response may be inversely related with the degree of CMV control. Remarkably, the incidence of post-transplant viremia was reduced among cases with high pre-transplant levels of NKG2C⁺ NK cells. The *NKG2C* genotypes distribution was comparable in KTR and healthy controls, and greater proportions of NKG2C⁺ cells were detected in *NKG2C+/+* than in *NKG2C+/del* patients. Yet, a trend towards increased *NKG2C+/del* and reduced *NKG2C+/+* frequencies associated to symptomatic infection was appreciated in both cohorts. Altogether, our results indirectly support that adaptive NKG2C⁺ NK cells are involved in the control of CMV in KTR.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-009

Impact of HCMV on native and modified HLA-I and consequences on gamma delta T cells activation

Layal Massara¹, Romain Marlin², Vincent Pitard², Jonathan Visentin², Gavin Wilkinson³, Ceri Fieldings³, Julie Déchanet-Merville²

¹ImmunoConcEpT, BORDEAUX, France

²Immunoconcept, CNRS UMR 5164, Bordeaux University, BORDEAUX, France

³Division of Infection and Immunity, School of Medicine, Cardiff University, CARDIFF, United Kingdom

The inhibition of HLA-I expression is used by HCMV to escape recognition by $\alpha\beta$ T cells. Native HLA molecules are normally expressed as α heavy chain/ beta2-microglobulin/antigenic peptide heterodimers on cell surface. In addition, "Modified HLA-I" have also been evidenced and are composed of an α -heavy chain alone, or associated only with either beta2-microglobulin or a peptide. These modified HLA-I are not recognized by $\alpha\beta$ TCR, but we showed that they were over expressed at the surface of HCMV-infected fibroblasts and potentially recognized by $\gamma\delta$ T cells. Our aim was to understand how HCMV induces the expression of modified HLA-I and how they influence T cell reactivity. We hypothesized that HCMV US2, US3, US6 and US11 genes involved in HLA-I downregulation could be involved in the overexpression of modified HLA-I. We used recombinant adenoviruses expressing each of the four US genes, and a mutant HCMV deleted for these 4 genes (CMV-DUS). We observed an induction of both native and modified HLA-I expression by the control adenovirus, and an inhibition of both forms by US2, US3 and US11. US6 inhibited native forms but maintains the expression of modified forms. When using CMV-DUS, infected cells expressed much more native and modified HLA-I than CMV-WT infected cells. Interestingly and in sharp contrast to $\alpha\beta$ T cells, $\gamma\delta$ T cell were activated to produce IFN γ when cultured with fibroblasts infected with CMV-WT, but not when fibroblasts were infected with CMV-DUS. These results indicate that HLA-I molecules regulate $\gamma\delta$ T cells through mechanisms that are under investigation in our team. The immune escape processes developed by CMV could thus promote $\gamma\delta$ over $\alpha\beta$ T cell response and explain the important response of $\gamma\delta$ T cells to the virus in immunosuppressed individuals.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-011

High latent viral loads in CD14+ monocytes associates with increased breadth and frequency of IFN γ secreting CMV specific T cells

Sarah Elizabeth Jackson, George Sedikides, Georgina Okecha, Emma Poole, John Sinclair, Mark Wills
University of Cambridge, Division of Infectious Diseases, Department of Medicine, CAMBRIDGE, United Kingdom

Human cytomegalovirus (HCMV) primary infection and periodic re-activation of latent virus is generally well controlled by T cell responses in healthy people of all ages. However in older people an increase in CMV DNA has been detected in urine, suggesting, that whilst still functional the CMV immune response is less able to control re-activation events, possibly due to immunomodulation as a consequence of lifelong carriage of the virus. Latent CMV infection is characterised by limited viral transcription, previously, we have reported that there is an IFN γ and cytotoxic CD4+ T cell response to UL138 and LUNA proteins, 2 latent associated transcripts (LATs), and also a cellular IL-10 CD4+ T cell specific response. IL-10 is a candidate to mediate immunomodulation of the immune response during ageing, to address whether long term carriage of HCMV changes the proportions of IL-10 and IFN γ secreting HCMV specific T cell populations and if altering the balance of IFN γ and IL-10 secreting T cells affects latent viral carriage, we analysed CMV specific responses in a large donor cohort aged 20-80 years. We measured T cell responses to 12 HCMV proteins (including 5 LATs) observing CD4+ and CD8+ IFN γ responses to all 12 proteins, with no increase in frequency or breadth of response with increasing age. IL-10-secreting CD4+ T cell responses were predominantly to LAT proteins, the magnitude and breadth of this response was also stable with age. While latent viral copy number did not increase with age, there

was a very significant positive association between the latent viral copy number and the magnitude and breadth of the IFN γ T cell response. This suggests that high latent viral loads may result in more frequent reactivation events leading to increased T cell activity and results in expansion of frequency and breadth of the CMV specific T cell response.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-012

Functional separation of IL7R α /KLRG1-defined CD8 $^+$ T cell populations in humans

Ester Remmerswaal¹, Michiel Van Aalderen¹, Pleun Hombrink², Anja Ten Brinke², Hanspeter Pircher³, Ineke Ten Berge¹, Rene Van Lier²

¹AMC, Experimental Immunology and Renal transplant Unit, AMSTERDAM, Netherlands

²Sanquin Research and Landsteiner laboratory, AMSTERDAM, Netherlands

³University Medical Centre Freiburg, Institute for Immunology, FREIBURG, Germany

During the acute phase of anti-viral CD8 $^+$ T cell responses in experimentally infected mice, short-lived effector cells (SLEC) have an IL-7R α ^{lo}KLRG1^{hi} phenotype, whereas IL-7R α ^{hi}KLRG1^{lo}, memory precursor effector cells (MPEC), are assumed to seed the memory compartment. It is unresolved if a subset division based on IL-7R α and KLRG1 would be useful to define functionally distinct cells within human virus-specific CD8 $^+$ T cell populations as they develop in the course of herpes virus infections.

We found that in healthy humans without any clinical signs of active viral infection not only MPEC and SLEC, but also CD8 $^+$ T cells with an IL-7R α ^{hi}KLRG1^{hi} phenotype (also referred to as double positive effector cells, DPEC) can readily be found in the circulation. In addition, a small population of early effector cells (EEC, IL-7R α ^{lo}KLRG1^{lo}) is present, which is the only population containing Ki-67 expressing cells. Notably, KLRG1-expressing cells are largely excluded from lymph nodes. The four populations defined by IL-7R α and KLRG1 differ markedly in transcription factor, granzyme and chemokine receptor expression. HCMV-specific populations contain low percentages of IL-7R α ^{hi}KLRG1^{lo} CD8 $^+$ T cells and are enriched for KLRG1-expressing T-bet^{hi} granzyme-B^{hi} cells. In contrast, EBV-specific T cells generally express more IL-7R α . EBV-specific cells that have low IL-7R α expression contain relatively low levels of T-bet and granzyme-B. Importantly, by studying primary infections we found that these differences between HCMV and EBV-specific responses were already installed early in the response.

Thus, combined analyses of IL-7R α and KLRG1 expression on human virus-specific CD8 $^+$ T cells can be used to separate functionally distinct subsets. As a non-cycling IL-7R α ^{lo}KLRG1^{hi} population is abundant in healthy humans, we conclude that this combination of markers expression not only defines short lived effector cells during the acute response but also stable effector cells that are formed and remain present during latent herpes infections.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-020

CMV specific CD8 $^+$ T cell responses in African children and adults

Amna Malik¹, Emily Adland¹, Leana Laker², Philippa Matthews¹, Pieter Jooste², Philip Goulder¹

¹University of Oxford, OXFORD, United Kingdom

²Kimberley Hospital complex, Paediatrics Department, Department of Paediatrics, KIMBERLEY, South Africa

Background

More than 90% of children in Africa are infected with CMV by the age of 12 months. This virus induces strong T-cell responses. These two facts provide the opportunity to study developing CMV specific CD8+ T-cell immunity through childhood. However little is known about the dominant CD8+ T-cell epitopes in African populations. In order to characterise the developing T-cell responses to CMV in children in Sub-Saharan Africa we here first sought to identify the immunodominant CMV epitopes within this populations.

Methods

257 HIV-infected ART-naïve adult and paediatric subjects of Sub-Saharan African origin were used in this study. We evaluated CTL responses in these individuals to overlapping peptides spanning the three most immunogenic CMV proteins, pp-65, IE-1 and IE-2, using IFN-g ELISpot assays. Peptide HLA-binding analysis and a bioinformatics prediction tool was used to predict optimal epitopes within all the reactive overlapping peptides. Validation of epitopes was undertaken in selected cases using HLA class I tetramer staining.

Results

We have identified >10 novel CMV-specific epitopes within CMV-pp65, IE-1 and IE-2. Five of these were also tested using peptide-MHC tetramers and confirmed to be bonafide epitopes restricted by the relevant HLA class I allele. One of the epitopes validated was an IE-2 epitope restricted by HLA*B*44:03 that induced consistently high CTL responses in individuals expressing HLA-B*44:03, in one individual accounting for 19% of their CD8 cells.

Conclusions

Multiple novel CMV-specific CTL epitopes, dominant within African populations, were defined and 5 confirmed by tetramer analysis. Each of the novel epitopes identified are critically important to the further analyses of CMV-specific CD8+ T-cell responses in African populations and are highly valuable in tracking changes to immune response in early childhood.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-022

B cell Phenotyping Before Renal Transplantation Associated With Cytomegalovirus Infection, But Not With Biopsy Proven Acute Rejection

Melissa Broeks, Wouter Lollinga, Weyel Abdulahad, Theo Bijma, Arjan Diepstra, Jacob Van den Born, Bouke Hepkema, Peter Heeringa, Jan-Stephan Sanders
UMCG, GRONINGEN, Netherlands

Background: Regulatory B cells could serve as regulators of immunological tolerance, and may modulate viral infections and rejection after transplantation. Our aim was to analyze B cell phenotyping as a measure of predicting biopsy-proven acute rejection (BPAR) and cytomegalovirus (CMV) infection.

Methods: Pre-transplantation samples of 110 renal transplant recipients were characterized for B cell subsets by flow cytometry. Risk for BPAR or CMV infection was determined with Cox regression using transitional CD24^{hi}CD38^{hi} and memory CD24^{hi}CD27⁺ B cells, and survival categorized with Log-rank test.

Results: B cell subsets were not significantly associated with presence of BPAR, and absence of BPAR was comparable between quartiles of B cells. However, risk for CMV infection increased by 10% (95% CI: 1.01 – 1.21; P = 0.04) for every extra percentage of CD24^{hi}CD38^{hi} B cells. Also, survival free of CMV infection was lower in the highest quartile of transitional CD24^{hi}CD38^{hi} B cells (P = 0.01), but did not differ for memory CD24^{hi}CD27⁺ B cells (P = 0.49).

Conclusions: A protective effect of transitional CD24^{hi}CD38^{hi} B cells on BPAR was not observed in this cohort, but incidence of CMV infection was higher. The latter could be due to the immune modulatory potential of Bregs, which may suppress protective host responses and increase susceptibility to infections. Additional studies have to provide further mechanistic insight on the regulatory mechanisms of B cells.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-026

Human Cytomegalovirus UL148 downregulates CD58 and demonstrates co-stimulation for CD8+ CTL against HCMV-, but not uninfected, targets

Eddie Chung Yern Wang¹, Mariana Pjechova², Evi Vlachava¹, Eva Ruckova², Ceri A Fielding¹, Rebecca J Aicheler³, Sepehr Seirafian¹, Luis Nobre⁴, Michael P Weekes⁴, Isa Murrell¹, Mihil Patel¹, Gavin Wilkie⁵, Richard J Stanton¹, Borivoj Vojtesek², Andrew Davison⁵, Paul J Lehner⁴, Gavin W Wilkinson¹, Peter Tomasec¹

¹Cardiff University, Division of Infection, School of Medicine, CARDIFF, United Kingdom

²Regional Centre for Applied Molecular Oncology (RECAMO), Masaryk Memorial Cancer, BRNO, Czech Republic

³Cardiff Metropolitan University, CARDIFF, United Kingdom

⁴University of Cambridge, CAMBRIDGE, United Kingdom

⁵University of Glasgow, GLASGOW, United Kingdom

Cytotoxic T-lymphocytes (CTL) cells kill virally infected cells by forming an immune synapse with their targets, through which activation and effector functions take place. The primary activation signal occurs through the T cell receptor (TCR) on CTL recognizing antigenic peptides presented in HLA-I molecules on targets, but a further component is the interaction between CD2 and the costimulatory cell adhesion molecule, CD58 (LFA-3). Using fibroblasts infected with a range of human cytomegalovirus (HCMV) deletion mutants and replication-deficient adenoviruses (rAds) expressing individual HCMV genes, we screened, with flow cytometry and proteomics, the U_L/b' region (absent in strain AD169) of the HCMV genome for regulators of CD58. A novel function encoded by low passage strains of HCMV, UL148, was identified, which mediated cell surface downregulation and intracellular retention of CD58. Functionally, we then defined the conditions at which CD2-CD58 interactions were most relevant for HCMV-specific CD8⁺ CTL using a HCMV deletion mutant missing UL148 and blockade with antagonistic anti-CD58 mAbs. Significant co-stimulation through the CD2-CD58 axis occurred over a narrow peptide range and could fully compensate for the reduction in CTL activation induced by the ~20-fold downregulation of HLA-I by HCMV-infected cells. This co-stimulation was, however, specifically directed against HCMV-infected, but not uninfected, targets. Thus, we propose UL148 as a novel evasion function targeted at CD8+ CTL, and demonstrate in turn that HCMV-specific CTL are finely tuned towards detecting cells infected with HCMV, even in the face of HCMV-encoded impairment of antigen processing and downregulation of HLA-I.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-028

Studies of innate-immune responses in the maternal-fetal interface reveal distinct mechanisms of defense against congenital HCMV and ZIKV infections

Yiska Weisblum, Esther Oiknine-Djian, Olesya Vorontsov, Ronit Haimov-Kochman, Zichria Zakay-Rones, Simcha Yagel, Amos Panet, Dana Wolf

Hadassah Hebrew University Medical Center, JERUSALEM, Israel

In view of the severe outcome associated with congenital HCMV infection, there is an urging need to better understand the innate mechanisms acting to limit trans-placental viral transmission. The placenta, given its critical function in protecting the developing fetus, has evolved effective, yet largely uncharacterized, innate immune barriers against invading pathogens. Recent studies, further facilitated

by the Zika virus (ZIKV) epidemic, have begun to uncover the importance of these local-placental innate immune responses in the defense against congenital infections. To gain a global insight into these earliest events of viral-tissue interplay within the authentic environment of the maternal-fetal interface, we have employed HCMV infection and genome-wide transcriptome analysis in maternal-decidual (the maternal aspect of the placenta) and chorionic villi (the fetal aspect of the placenta) tissues, maintained as integral 3D organ cultures. We further compared the innate tissue-response patterns following parallel infections with HCMV and ZIKV. Notably, HCMV and ZIKV induced divergent innate responses in both maternal and fetal-derived tissues. While HCMV response was predominated by upregulation of immune-cells activation, proliferation, and cell trafficking pathways, these pathways were not significantly altered by ZIKV. Specifically, interferon (IFN) γ , that was profoundly upregulated by HCMV, was not affected by ZIKV, which rather induced the expression of type I and type III IFNs. Of note, chorionic-villi responses to ZIKV were distinctively enriched for apoptosis, cell-death, and necrosis molecular functions. Interestingly, focusing on the most profoundly upregulated genes in the HCMV-infected decidual tissues, we have identified the activation of previously unexplored anti-HCMV innate immune mechanisms. We are currently characterizing their biological function in the defense against HCMV transmission. These studies provide a novel insight into front-line placental tissue innate responses which could mediate the outcome of congenital infection.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-030

Human foetal hematopoietic stem and progenitor cells (HSPC) generate invariant HCMV-reactive $\gamma\delta$ T cells

David Vermijlen¹, Paola Tieppo¹, Deborah Gatti¹, Françoise Gosselin², Glenn Goetgeluk³, Arnaud Marchant¹, Catherine Donner², Bart Vandekerckhove³

¹ULB (Université Libre de Bruxelles), BRUSSELS, Belgium

²Hôpital Erasme, BRUSSELS, Belgium

³Ghent University, GHENT, Belgium

Although there are common characteristics among $\gamma\delta$ T cells, it is clear that $\gamma\delta$ T cells do not represent a homogenous population of cells with a single physiological role. We have shown that in congenital human cytomegalovirus (HCMV) infection, but not in adult HCMV infection, $\gamma\delta$ T cells expressing (semi)invariant germline-encoded T cell receptors (TCR) dominate the $\gamma\delta$ T cell repertoire. We hypothesized that these 'early' $\gamma\delta$ T cell subsets are made by specific haematopoietic stem and precursor cells (HSPC) present in foetal life.

In an *in vitro* T cell development system, human foetal (gestation 28-30 weeks, no infection) HSPC-derived $\gamma\delta$ T cells and term-delivery (> 37 weeks gestation, no infection) HSPC-derived $\gamma\delta$ T cells were enriched for different γ and δ chain combinations as determined by flow cytometry. A more detailed analysis of the complementary determining region-3 (CDR3) γ and CDR3 δ repertoire by high-throughput sequencing indicated that foetal HSPC-derived $\gamma\delta$ T cells, in contrast to term-delivery HSPC-derived $\gamma\delta$ T cells, were highly enriched for particular invariant germline-encoded CDR3 sequences that are found in congenital HCMV infection. Furthermore, we could reprogram term-delivery towards a foetal-like generation of $\gamma\delta$ T cells by transducing term-delivery HSPC with an RNA-binding protein. Together, these data indicate that foetal HSPC generate $\gamma\delta$ T cells expressing germline-encoded invariant $\gamma\delta$ TCRs programmed to respond to HCMV infection.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-034

HCMV- specific Antibody Mediated NK- cell Activation and Neutralization in Lung Transplant Recipients

Hannes Vietzen, Irene Görzer, Elisabeth Puchhammer-Stöckl
Medical University of Vienna, Department of Virology, VIENNA, Austria

Background:

Human Cytomegalovirus (HCMV) leads to increased morbidity in lung-transplant recipients (LTR). The HCMV specific antibody (AB) response limits virus spread in LTR but its fine specificity is poorly understood. We analyzed the post-transplant development of HCMV neutralization and NK-cell activation in HCMV seropositive recipients (R+) and its relation to HCMV replication. In addition, we assessed the impact of specific IgG1 AB constant heavy chain variants in the CH1 region on these HCMV specific AB responses.

Methods:

A total of 32 R+ LTRs were included amongst which 21 had no HCMV replication and 11 had detectable HCMV replication within the first year post-transplantation. From each patient, plasma samples were obtained within one week of transplantation, 3 months later after stop of antiviral prophylaxis, and at time of HCMV replication or at a similar time point if no virus replication occurred. Patients were genotyped for the IgG1 genetic marker (GM) 3/17 variants. In each sample NK-cell activation was analyzed in a bioluminescent reporter-assay with FcγRIIIa receptor-expressing effector cells, and the 50% effective concentration (EC50) was determined. The 50% neutralization titer (NT50) was determined by neutralization ELISA.

Results:

Both, HCMV neutralization and HCMV-specific NK-cell activation were lowest 3 months post TX, and overall significantly increased thereafter (both $P=0.0001$; Mann-Whitney test). Individuals encoding for GM3/17 showed significantly better NK-cell activation compared to patients with GM3/3 and GM17/17 variants, especially early post-transplantation and in the later follow up ($P=0.0001$ and 0.0014 ; Mann-Whitney test) while there was only limited association with neutralization capacity. Clearly lower neutralization at 3 months post transplantation, was observed in patients with further HCMV replication ($P=0.015$, Mann-Whitney test).

Conclusion

The GM3/17 variant is significantly associated with better HCMV specific NK-cell activation after transplantation. The neutralizing AB capacity at 3 months post-transplantation, after gancyclovir prophylaxis, is associated with further HCMV replication.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-038

A modified protein in situ hybridization method allows screening of genetic variants of glycoprotein B AD4 mutants for its immunogenicity

Martina Schneider¹, Ramona Hartl¹, Norman Kilb², Tobias Herz², Guenter Roth², Michael Mach³, Christoph Steininger¹

¹Medical University of Vienna, Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, VIENNA, Austria

²Albert-Ludwigs University of Freiburg, Center for Biological Systems Analysis (ZBSA); Institute for Biology III, FREIBURG, Germany

³Friedrich-Alexander-University of Erlangen-Nuernberg, Institute of Clinical and Molecular Virology, ERLANGEN, Germany

Background: Until now there is no effective vaccine against Cytomegalovirus (CMV), although it is of major medical importance. CMV is a highly variable pathogen and this diversity was correlated with its pathogenicity.

Method: We established a method that allows us to study genetic variants of CMV with a modified protein *in situ* hybridization method. The gene glycoprotein B discontinuous neutralizing-epitope AD4

and the mutant variants are amplified via PCR from a DNA template, translated to protein directly with an *in vitro* translation system and immobilized via a capture tag at the C-terminal end on modified glass slides. Screening with different patient sera and visualization of this immune reaction allows the evaluation of the immune response of serum antibodies to the genetic variant.

Results: This was a proof-of concept study and we evaluated different genetic variants of glycoprotein B discontinuous neutralizing-epitope AD4. The result confirms the importance of the tyrosine (Y) at position 364 and a lysine (K) at position 379 from sera of infected individuals. Primary infections and CMV negative do not show an IgG-antibody response towards AD4 or its mutants.

Conclusion: This method allows the screening of genetic variants for their immunogenicity.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-041

Characterization of immunological reaction to CMV in super healthy elderly

Sara P.H. Van den Berg¹, Nening M. Nanlohy¹, Lisa Beckers¹, Renée Van Boxtel¹, Jose A.M. Borghans², Debbie Van Baarle¹

¹National Institute for Public Health, Center Infectious Diseases, BILTHOVEN, Netherlands

²University Medical Center Utrecht, UTRECHT, Netherlands

The immune system controls CMV-infection and prevents viral dissemination in healthy elderly. However, there are large inter-individual differences in the height of anti-CMV IgG levels or T cell responses to CMV in elderly. This might be influenced by frail health status or comorbidities. In this study, we investigate the CMV immune response in strict defined healthy elderly (60-80 years), to better understand and characterize the immune response to latent CMV-infection. 30 CMV+ elderly persons who fulfilled the health criteria (f.e. no medicine-use, allergies, comorbidities or history of severe infectious diseases) were studied. CMV-specific cells were identified with 8 different MHC-I dextramers for immunodominant CMV-epitopes representing 7 different HLA types covering 95% of the European population. CMV-specific cells for one or more epitopes were detected in 22 out of 30 CMV-positive individuals. The percentage of CMV-specific cells (mean 4,5% range 0,19%-23,5%) varied between individuals, reaching over 10% of total CD8 T cells for one CMV-epitope in some individuals (n=4). A higher percentage CMV-dextramer cells positively correlated with a more differentiated phenotype (percentage T_{EMRA} cells). The IFN γ response to CMV after stimulation with peptide pools pp65, IE1 and UL55 and CMV lysate also showed great inter-individual differences between donors. Interestingly, the height of the response to these three immunodominant peptide pools correlated with each other, suggesting that this response can be taken as a measure of the total CMV-response. Moreover, the IFN γ response to the CMV-peptide pools also positive correlated with the anti-CMV IgG level (R^2 : 0,61, $p < 0,0001$). In conclusion, in these super healthy elderly different immune parameters behave in a similar manner, suggesting synergy between the different arms of the immune system. However, there is great inter-individual variation, suggesting that the level of immunity to CMV does not per se correspond to their health.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-042

Are maternal HCMV-specific CD8+ T cells at the maternal-fetal interface able to cross-react with fetal HLA-C?

Anita Van der Zwan, H. Van den Heuvel, P.M.W. Van der Meer-Prins, P. Van Miert, S. Heidt, F.H.J. Claas

CMV infection can lead to the induction of HLA-restricted virus-specific CD8+ T cells that often cross-react with allogeneic HLA molecules. This so called heterologous immunity may impact the outcome of organ transplantation as the presence of activated or memory T cells, reactive against the foreign HLA antigens of the donor organ, will lead to a more vigorous allo-immune response and possibly graft rejection.

HCMV infection is a major cause of severe congenital disease. About 1 out of 150 babies are born with congenital CMV infection that may result in hearing loss and developmental disabilities. HCMV infects many cell types in the placenta, including decidual stromal cells (DSC), decidual macrophages and extravillous trophoblasts (EVT). Uncontrolled placental viral infections are accompanied by a pro-inflammatory milieu that can alter the stability and activation status of effector T cells.

Recently, we showed that HLA-A/B-restricted HCMV-specific CD8+ effector and memory T cells are abundantly present in the decidua. These CD8+ T cells, which are meant to protect the mother, may in theory cross-react with the paternal HLA antigens of the foetus leading to pregnancy complications. However, the only polymorphic allogeneic HLA molecule expressed by EVT, and a potential ligand for decidual CD8+ T cells, is HLA-C.

In the present study, we assessed whether virus-specific CD8+ T cell clones, known to cross-react with allogeneic HLA, are also able to cross-react with HLA-C. Extensive experiments showed that, in contrast to HLA-A and HLA-B, HLA-C seems to not be a target of these virus-specific T cells. Whether maternal HLA-C-restricted HCMV-specific T cells cross-react with fetal HLA-C remains to be established.

It is of high clinical relevance to understand how these maternal CD8+ T cells integrate the antithetical need of maternal-fetal tolerance and anti-viral immunity to establish a healthy pregnancy.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-044

Human cytomegalovirus-specific memory T-cell response and its correlation with virus transmission to the fetus in pregnant women with primary infection

Chiara Fornara, Irene Cassaniti, Maurizio Zavattoni, Milena Furione, Kodjo MG Adzasehoun, Giuditta Comolli, Fausto Baldanti

Fondazione IRCCS Policlinico San Matteo, Molecular Virology Unit, Microbiology and Virology Department, PAVIA, Italy

Background: Primary Human cytomegalovirus (HCMV) infection during pregnancy is the major cause of congenital viral infection. The HCMV-specific T-cell response may have a role in the prevention of virus transmission to the fetus.

Methods: HCMV-specific memory T-cells were investigated in 44 pregnant women (15 of whom transmitted the infection to the fetus) within 1-2 months after onset of primary infection. For comparison, 8 pregnant women with remote infection were analyzed. PBMC were stimulated for 12 days with overlapping 15-mer peptide pools of HCMV proteins immediate early (IE)-1, IE-2 and phosphoprotein (pp) 65, and subsequently re-stimulated with the corresponding peptide in a cultured-ELISPOT assay.

Results: In remote infections no significant difference was observed among T-cell responses to pp65, IE-1 and IE-2. Instead, in primary infection, the pp65-specific T-cell response was significantly greater with respect to IE-1 and IE-2 ($p < 0.05$). However, the response to all three proteins was significantly lower in primary infection with respect to remote infection ($p < 0.05$ for pp65, $p < 0.01$ for IE-1 and IE-2). In primary infection, expandable T-cells directed to pp65 and, when detectable, to IE-1 were predominantly CD4⁺. Strikingly, the response to pp65 was significantly lower ($p < 0.01$) in women transmitting the infection to the fetus. A cultured ELISPOT response > 20 was associated with an odds ratio of 7.14 (95% CI 1.65 to 30.89) for non-transmission of the virus to the fetus.

Conclusions: Determination of pp65-specific expandable memory T-cells in pregnant women with primary HCMV infection is a promising tool to assess the risk of HCMV fetal transmission.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-051

Hyperechogenic bowel and congenital CMV infected fetuses

Liliana Gabrielli¹, Maria Paola Bonasoni², Giuliana Simonazzi³, Maria Grazia Capretti⁴, Francesca Cervi³, Donatella Santini⁵, Angela Chierighin⁶, Giulia Piccirilli⁶, Claudia Pavia⁶, Gabriele Turello⁶, Diego Squarzoni⁶, Tiziana Lazzarotto⁶

¹St. Orsola-Malpighi University Hospital, Operative Unit of Clinical Microbiology, BOLOGNA, Italy

²O.U. of Pathology, St. Maria Nuova Hospital, REGGIO EMILIA, Italy

³O.U. of Obstetrics and Gynecology, University of Bologna, BOLOGNA, Italy

⁴O.U. of Neonatology, St. Orsola-Malpighi University Hospital, BOLOGNA, Italy

⁵O.U. of Pathology, St. Orsola-Malpighi University Hospital, BOLOGNA, Italy

⁶O.U. of Microbiology, Policlinico S. Orsola-Malpighi, University of Bologna, BOLOGNA, Italy

Prenatal ultrasound findings in fetuses with CMV infection may include hyperechogenic bowel. The objective of our study was to find a possible pathophysiology underlining hyperechogenic bowel in congenital CMV infected fetuses.

We examined small and large intestines and pancreas in 8 fetuses at 22 weeks of gestation with congenital CMV infection. Fetal diagnosis of CMV infection was based on CMV positivity in amniotic fluid by culture and Real Time PCR. Ultrasound findings showed four fetuses with hyperechogenic bowel and two of them with brain abnormalities. The other 4 fetuses had no ultrasound anomalies. Serial sections of duodenum, jejunum, ileum, large bowel and pancreas were submitted for histological examination. Immunohistochemistry for CMV and lymphocytic infiltrate were also performed.

In the 4 fetuses with hyperechogenic bowel, macroscopic autopsy showed dilatation of the distal intestine, especially ileum and large bowel. In addition, meconium appeared thickened and distally localized. Microscopic examination showed intestinal ganglioneuritis with CMV positive cells in the intestinal ganglia surrounded by a lymphocytic infiltrate mainly composed of cytotoxic T CD 8. CMV positive ganglion cells were observed only in the Auerbach's myenteric plexus through all different intestinal regions. Pancreas was grossly normal, however at histology, epithelial cells were CMV positive, mostly surrounded by T CD 8 lymphocytes. Moreover, meconium granules within the enterocyte cytoplasm facing the intestinal lumen were observed. These may represent indirect signs of reduced intestinal peristalsis. In the 4 fetuses with no hyperechogenic bowel, macroscopically the intestine was not dilated and intestinal sections showed either no CMV cells or inflammatory infiltrate. However, pancreatic histology showed CMV positive cells and lymphocytic infiltrate similarly observed in fetuses with hyperechogenic bowel.

In conclusion, hyperechogenic bowel probably can be explained primarily as reduced intestinal motility due to CMV ganglioneuritis in the Auerbach's myenteric plexus instead of impairment in pancreatic enzymatic secretions.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-003

US16 Protein of Human Cytomegalovirus Contributes to Virus Tropism for Endothelial and Epithelial Cells by Regulating Virion Pentameric Complex Content

Anna Lugini¹, Noemi Cavaletto¹, Federica Galati¹, Federica Galliano¹, Grigoris Kokkoris¹, Stefania Raimondo², Stefano Geuna², Giorgio Gribaudo¹

¹Department of Life Sciences and Systems Biology, UNIVERSITY OF TORINO, TORINO, Italy

²Department of Clinical and Biological Science, TORINO, Italy

The US12 gene family of HCMV encodes a set of 10 multi transmembrane proteins whose specific functions have not been established yet. While inactivation of individual US12 gene members in laboratory strains of HCMV does not affect viral replication in fibroblasts, we observed that disruption of US16, US18, US20, and US21 in the TR clinical strain prevents viral growth in other cell types, thus suggesting a role in HCMV cell tropism. Recombinant viruses with deletion or functional inactivation of the US16 gene failed to express IE, E, and L viral proteins in endothelial, epithelial and dendritic cells, thus indicating a defect of the US16-deficient viruses which occurs prior to IE gene expression. This defect was due to inefficient virus entry. In fact, ultrastructural analysis of infected endothelial cells revealed that US16-null particles were internalized and accumulated in vacuoles shortly after infection but were not released from endocytic vesicles. A link between pUS16 and the pentameric gH/gL/UL128/UL130/UL131 complex, required for infection of endothelial and epithelial cells, was then demonstrated by the absence of pUL128 and pUL130 in purified virions released from fibroblasts infected with genetically US16-deficient viruses, and by the colocalization and interaction of pUS16 and pUL130 in infected fibroblasts. Deletion of the C-terminal tail of pUS16 reproduced the defective phenotype of US16-null viruses. However, assembly and trafficking of the pentameric complex to cVAC and cVAC formation were not affected by the lack of either pUS16 or its C-terminal tail, thus suggesting that pUS16 regulates the tropism of HCMV by dictating a late stage of viral maturation in a gH/gL form-specific manner. Identification and characterization of determinants of viral tropism, like US16, will advance our understanding of the molecular mechanisms of HCMV-related pathogenesis and may lead to the design of new antiviral strategies to exploit these functions.

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380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-004

US28-induced changes in host factors during HCMV latency

Elizabeth Elder, Benjamin Krishna, James Williamson, Paul Lehner, John Sinclair
University of Cambridge, Department of Medicine, CAMBRIDGE, United Kingdom

The G-protein coupled receptor US28 is one of a small number of HCMV genes expressed during latency, and its expression is required for latency establishment. In order to understand the mechanism underlying this, a whole-cell proteomic screen was conducted in monocytic cells stably expressing wild-type US28 (US28-WT) or a signalling-defective (but correctly folded) US28 mutant (US28-R129A). In the screen, two Aim2-like receptor (ALR) family proteins, IFI16 and MNDA, were significantly downregulated in the presence of wild type US28. ALR proteins have previously been linked to inflammasome activation and Type I interferon production and, interestingly, we have obtained preliminary data which suggests that the expression of US28 abrogates DNA-stimulated type I interferon production in monocytic cells. Although such a role has been proposed for IFI16 in the context of HCMV lytic infection in fibroblasts, our observations hint at a completely novel role for MNDA and IFI16 in sensing the latent viral genome.

Additionally, we have found that the transcriptional repressor CTCF is greatly upregulated in the presence of US28-WT but not US28-R129A. Further analyses show that CTCF binds and represses the MIEP and that knock-down of CTCF results in increased IE expression in latently infected cells. Furthermore, monocytic cells infected with a US28 deletion virus, which fail to establish latency, expressed IE at a lower level when CTCF was overexpressed.

This work helps to further our understanding of the host factors required for the establishment of HCMV latency.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
B-PP-006

Maturation of Langerhans-type dendritic cells alters human cytomegalovirus gene expression, progeny production and reactivation from latency in a stimulus-dependent manner.

Laura Hertel, Roxanne Coronel, Melissa Galinato, Olabisi Osunmakinde, Lucia Dalle Ore
Children's Hospital Oakland Research Institute, OAKLAND, United States of America

Human cytomegalovirus (CMV) transmission is thought to occur by contact between virions and oronasal mucosae, whose outer layers are exclusively comprised of epithelial cells and Langerhans-type dendritic cells (LC). Being the first innate immune cells to encounter incoming CMV virions, LC are thus poised to play important roles in host defenses against this virus. We show that although virion entry and nuclear deposition of viral genomes occurs in a large majority of immature LC (iLC), expression of the viral immediate-early genes occurs only in 2-5% of cells. Despite this, iLC produce 10-fold higher amounts of viral progeny than mature LC (mLC) obtained by stimulation with GM-CSF, LPS, CD40L and fetal bovine serum (FBS), even though mLC populations contain larger numbers of infected cells (10-20%). This suggests that CMV replication in LC undergoes at least two blocks: one acting early and restricting infection onset in both iLC and mLC, and one acting later, reducing viral yields in mLC. Although exposure to pro-inflammatory cytokines or to CD40L alone increased the proportion of infected cells, neither stimulus could fully remove the block to infection onset. While CD40L and FBS alone enhanced viral progeny production, stimulation with LPS alone or combined with CD40L and FBS repressed it, suggesting that LPS exerts dominant negative effects. Finally, iLC supported viral reactivation without the need for maturation, but reactivation rates were increased in populations exposed to pro-inflammatory cytokines. Together, these data show that immature LC, such as those found in the oral cavity of healthy individuals, are fully capable of supporting CMV acquisition and transmission without the need for maturation. Both processes, however, can be modulated by LC encounters with different types of extracellular stimuli.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
B-PP-013

Subcellular localization of pp65 is a critical determinant for human cytomegalovirus productivity

Bodo Plachter, Christine Zimmermann, Caroline Sauer, Nicole Büscher
University Medical Center Mainz, Institute for Virology, MAINZ, Germany

The tegument protein pp65 (pUL83) of human cytomegalovirus (HCMV) is one of several viral polypeptides that are involved in the subversion of cellular antiviral defense functions. It is immediately translocated to the nucleus of permissive cells at the time of infection. Despite its strong nucleophilic nature, pp65 is preferentially found in the cytoplasm at late stages of permissive infection, primarily accumulating at cytoplasmic viral assembly sites. We have previously shown that a nuclear retention phenotype of pp65 was associated with limited viral productivity. It remained unclear, however, if nuclear accumulation of pp65 or its limited availability in the cytoplasm was responsible for that phenotype. In this study we addressed the impact of the subcellular distribution of pp65 on productive HCMV infection, using the TB40/E for analysis. This strain expresses low levels of pp65 compared to laboratory strains, shows limited productivity in both HFF and ARPE-19 epithelial cells and displays little pp65 cytoplasmic accumulation at late stages of infection. We first asked if overexpression of pp65 from a second copy of the UL83 gene in a mutant virus would lead to enhanced cytoplasmic expression and, thereby, increased productivity. However, the preferential nuclear accumulation of pp65 was not reverted despite its overexpression and viral productivity was reduced rather than enhanced. In a second approach, we generated a TB40/E mutant in which the nuclear localization signals of pp65 were mutated. This virus surprisingly showed enhanced viral DNA replication and virus release from both infected HFF and ARPE-19, compared to the parental TB40/E strain. The data showed (i) that it was indeed nuclear accumulation of pp65 that was repressive to the release of viral progeny. The results also revealed (ii) that the cytoplasmic accumulation of pp65 is a critical determinant for the level of HCMV DNA replication

and progeny production.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-015

L-proline promotes human Cytomegalovirus replication via activation of DNA Damage Response Pathways

Huanhuan Leah Cui¹, Jiri Bartek², Cecilia Soderberg Naucner¹

¹Karolinska Institute, STOCKHOLM, Sweden

²Danish Cancer Society Research Centre, Genome Integrity Unit, COPENHAGEN, Denmark

Amino acids have been implicated in metabolic reprogramming of human cytomegalovirus (HCMV) infected cells, examples include increased glucose uptake for fatty acid biosynthesis or upregulation of glutamine for ATP production. Another source of metabolite that can be catabolised for supply of ATP is L-proline, however the role of L-proline in HCMV infection is unknown. In mitochondria, proline can be synthesised from glutamine or urea derived ornithine via the intermediate pyrroline-5-carboxylate (P5C). P5C is converted to proline by pyrroline-5-carboxylate reductase (PYCR), an enzyme that exists in three isoforms: PYCR1, PYCR2, and PYCRL. Here, we show that in fibroblasts (MRC5) infected with the VR strain, all PYCR isoforms are significantly increased in both mRNA and protein levels and was evident already at 24 hours post-infection. However, in endothelial cells (HUVEC), the increase was delayed and can only be observed at 72 hours post-infection. In fibroblast cells, the intracellular L-proline concentration was 2-fold higher in infected cells when compared to uninfected cells. The addition of exogenous proline significantly increased both immediate early (IE) and late protein PP28 levels, and resulted in a significant increase in viral replication. Furthermore, knockdown of PYCR isoforms using siRNA reduced the number of infected cells by approximately 50%. Depletion of the cellular proline pool by treatment with Halofuginone also significantly reduced IE abundance; this effect was reversible with the addition of exogenous proline. We further found that proline is valuable for HCMV infection by mediating suppression of reactive oxygen species and more importantly, through the activation of DNA damage responses (DDR). Thus, HCMV induces an L-proline dependent DDR activation, which involves activation of both the ATM and ATR signalling pathways. We conclude that HCMV exhibits an increased proline dependency and may be manageable by targeting metabolic pathways involved in proline biosynthesis.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-017

Targeting Pol I through CX5461, a specific Pol I inhibitor, or Pol I siRNA, affects HCMV transcription, not virus production

Ourania N. Kostopoulou¹, Vanessa Wilhelmi¹, Sina Raiss¹, Sharan Ananthaseshan¹, Mikael Lindström², Jiri Bartek², Cecilia Söderberg-Naucner¹

¹Karolinska University Hospital, Department of Medicine, STOCKHOLM, Sweden

²Karolinska Institute, Dep. of Medical Biochemistry and Biophysics, Science For Life Laboratory (MBB), SOLNA, Sweden

The nucleolus is a non-membrane-bound nuclear organelle found in all eukaryotes. Its assembly is triggered by activation of RNA polymerase I-mediated transcription and regulated in a cell cycle-dependent manner in mammalian cells. Many viruses including human cytomegalovirus (CMV) can

specifically target the nucleolus to facilitate viral transcription and translation. CMV infection results in increased protein synthesis and an enlarged nucleoli. CX-5461 targets the SL1 transcription factor of the Pol I complex and induces autophagy and senescence in a number of solid tumor cell lines, but inhibits neither Pol II transcription of mRNA nor the synthesis of DNA or proteins. We examined the antiviral effect of CX5461 compared with the anti-CMV drug Ganciclovir. In order to confirm that CX-5461 is working as a Pol I inhibitor, we assessed its effects on FUr d incorporation (a fluorine-conjugated uridine analogue, which reflects that rRNA comprises more than 80% of cellular RNA) and production of 47S transcripts. These parameters were both significantly reduced. Normal cells (fibroblasts) were pretreated with CX5461 and Ganciclovir and then infected with CMV strain VR1814. The potential antiviral effect of CX5461 was examined on immediate early (IE), pp65, gB transcripts and IE and pp65 protein levels as well as virus production. IE transcripts were quantified by qPCR and were significantly reduced by both drugs. Immunofluorescence and western blot analyses confirmed significantly reduced levels of IE and pp65 proteins in infected cells treated with CX5461. Experiments using siRNA to Pol I also demonstrated significantly reduced levels of viral transcripts and IE protein levels. However, production of infectious virus was unaffected at 7 days post infection. To conclude, we provide evidence to suggest a potentially important role of Pol I, direct or indirect, in the early transcription of HCMV, yet no essential for later virus production.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-018

The oncomodulatory role of HCMV-encoded chemokine receptor US28 in glioblastoma

Tian Shu Fan¹, Raimond Heukers¹, Raymond De Wit¹, Jeffrey Van Senten¹, Laura Smits-de Vries¹, Tonny Lagerweij², Rahbar Afsar³, Siderius Marco¹, Vischer Henry¹, Würdinger Tom², Cecilia Söderberg-Nauclér³, Smit Martine¹

¹VU University Amsterdam, Medicinal Chemistry, AMSTERDAM, Netherlands

²VUMC Cancer Center Amsterdam, AMSTERDAM, Netherlands

³Karolinska Institute, STOCKHOLM, Sweden

Human cytomegaloviruses (HCMVs) have been found to infect cancer (stem) cells (CSCs) in a highly efficient manner, thereby stimulating self-renewal proliferation. HCMV encodes for four known viral G protein-coupled receptors (GPCRs); US28, US27, UL33, and UL87, which show high homology to human chemokine receptors. Constitutive activation of US28 has been associated with an oncogenic phenotype via interference with cellular signaling and stimulation of tumor growth via angiogenic and proliferative pathways. Moreover, HCMV-encoded US28 has been found in glioblastoma patient material with localization particularly around the vascular niches. In order to determine the role of HCMV-encoded US28 in glioblastoma, we have investigated US28-mediated stimulation of signaling and proliferation *in vitro* in both 2D and 3D cultures of differentiated glioblastoma cells and primary glioma cells, and an orthotopic *in vivo* glioblastoma model in mice. US28 correlated with the activity of stemness regulators and US28 expression stimulated glioblastoma growth *in vitro* and *in vivo*. These observations emphasize the oncomodulatory role of US28 in glioblastoma and potentially other HCMV-associated malignancies.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-019

Inhibitors of the autophagy-initiating protein kinase Ulk1 interfere with HCMV replication

Thomas Stamminger, Patrick König, Adriana Svrlanska, Sabine Feichtinger

Research conducted during the last few years revealed that the cellular protein kinase Ulk1 exerts critical regulatory functions at the intersection of autophagy, innate immunity and inflammatory disorders. This protein was first described as autophagy-initiating protein kinase, however, recent evidence suggests that Ulk1 is an important component of different protein complexes involved in pathogen recognition. Furthermore, Ulk1 not only controls the onset of macroautophagy and mitophagy but also fine-tunes and negatively regulates inflammatory processes thus preventing immunopathology. We observed that Ulk1 is strongly upregulated after infection of primary human fibroblasts with human cytomegalovirus (HCMV). In addition, we detected an enhanced phosphorylation of Ulk1 at various serine residues which are typically targeted by the cellular AMP-activated protein kinase (AMPK), a metabolic stress response kinase. Inhibition of AMPK activity reversed this HCMV-induced modulation of Ulk1 which correlated with an impairment of viral replication. Evidence for a proviral role of Ulk1 was also obtained by generating primary human fibroblasts with a stable Ulk1-knockdown which revealed a profound growth defect of HCMV in the absence of Ulk1. Furthermore, small molecule inhibition of Ulk1 kinase activity strongly interfered with HCMV replication. Collectively, these data suggest that Ulk1 may serve as a novel target molecule for antiviral therapy. Moreover, viral dysregulation of Ulk1 may contribute to immunopathology frequently observed in connection with HCMV infection.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-023

Differences in growth properties among two human cytomegalovirus glycoprotein O genotypes

Irene Goerzer¹, Julia Kalser¹, Barbara Adler², Elisabeth Puchhammer-Stöckl¹

¹Medical University of Vienna, Department of Virology, VIENNA, Austria

²Max von Pettenkofer-Institut, Virology, MUNICH, Germany

Glycoprotein O (gO) of the human cytomegalovirus (HCMV) is the critical subunit of the envelope trimer gH/gL/gO as it interacts with platelet-derived growth factor alpha receptor upon fibroblast entry, and triggers gB-mediated fusion for fibroblast and epithelial cell infection. Eight genotypes (GT) of the highly polymorphic gO gene are described, yet it is unclear whether the distinct GTs differ in their function. Thus, we aimed to elucidate potential differences between two highly diverse gO GTs in an otherwise genetically identical HCMV background. Therefore, resident gO GT1c sequence of HCMV strain TB40-BAC4-luc was entirely replaced by GT4. In addition, two conserved gO cysteines involved in gH/gL/gO stabilisation (GT1c-C218 and C343) were mutated to serine in both genotypes. All these gO mutants were investigated for their growth properties in fibroblasts and epithelial cells. GT4 displayed a significantly enhanced infectivity for both cell types and higher virus release upon replication in epithelial cells when compared to GT1c. The cysteine mutants GT1c-C218S and GT4-C216S showed comparable infection efficiencies as their parental strains. For the GT1c-C343S mutant, infection capacity was moderately impaired, while the infection efficiency of GT4-C336S was strongly reduced for both fibroblasts and epithelial cells. These findings suggest that different gO genotypes might affect the infection efficiency also in vivo and their function may be differentially susceptible to mutations which may have important implications for virus host transmission.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-024

Genotypic analysis of HCMV polymorphism in congenital infections to predict the impact of HCMV disease severity in newborns/infants

Ganna Galitska¹, Valentina Dell'Oste¹, Matteo Biolatti¹, Marco De Andrea¹, Agata Leone², Enrico Bertino², Santo Landolfo¹

¹University of Turin, Department of Public Health and Pediatric Sciences, TURIN, Italy

²Department of Public Health and Pediatric Sciences, Neonatology Unit, TURIN, Italy

Human Cytomegalovirus (HCMV) is the leading cause of birth defects related to an infectious agent. Prevention and clinical interventions for HCMV disease are limited by the absence of an effective vaccine, as well as resistance to antiviral drugs and therapeutic neutralizing antibodies. Recently, it has been proposed that HCMV disease and pathogenesis may be affected by the genetic diversity of the virus, exceptionally high both within and between hosts. Virus load, replication rate and clinical sequels of the congenitally infected infants may thus result from HCMV genetic polymorphism. Although it is known that HCMV can extensively hijack cellular genes that may contribute to its immune evasion capability and that the virus is polymorphic among hosts, the source of genomic variability and the biological relevance remain unresolved.

In this study, we aimed to characterize HCMV clinical isolates obtained from newborn infants diagnosed with congenital or postnatal HCMV infection for the genomic variability of specific genes encoding potential virulence factors or involved in antiviral drug resistance and to identify correlations between viral genotypes, phylogeny, *in vitro* growth properties and clinical sequels. *In vitro* analysis of the virions by Focus Expansion Assay (FEA) indicated different patterns of replication, that enabled us to group them into three categories: fast-, intermediate-, and slow-replicating strains. Interestingly, the *in vitro* replication scores correlate with the patients' viremia titres at the time of sampling. Moreover, preliminary results obtained from the alignment of the HCMV genome sequences and compared to the reference strain Merlin (NC_006273) revealed that divergent clades can be outlined within the viral population, indicating that heterogeneous viruses exist. This study may contribute to understanding the clinical significance of viral genetic diversity in predicting the impact of virulence factor variability on congenital HCMV disease severity.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-027

The DNA Cytosine Deaminase APOBEC3G is upregulated during Human Cytomegalovirus infection

Sara Pautasso¹, Ganna Galitska¹, Valentina Dell'Oste¹, Matteo Biolatti¹, Francesca Gugliesi¹, Gloria Griffante¹, Marisa Gariglio², Marco De Andrea¹, Santo Landolfo¹

¹University of Turin, Department of Public Health and Pediatric Sciences, TURIN, Italy

²Department of Translational Medicine, University of Eastern Piedmont, NOVARA, Italy

The Apolipoprotein B Editing Enzyme Catalytic subunit 3 (APOBEC3) is a family of DNA cytosine deaminases that mutate and inactivate viral genomes by single-strand DNA editing, with important roles in innate immunity and cancer. It has been demonstrated that APOBEC3 interfere with viral replication of RNA and DNA viruses, but its role during Human Cytomegalovirus (HCMV) replication has never been investigated. APOBEC3 is able to counteract viral replication increasing mutation rates in viral genome. On the other hand, HCMV displays high variability limited to distinct parts of the genome. In this study we analysed the impact of HCMV infection on APOBEC3 expression and activity. We observed that APOBEC3G is upregulated by HCMV infection in primary Foreskin Human Fibroblast (HFF) at both mRNA and at protein level. However, although strongly induced, the specific knockdown of APOBEC3G does not negatively affect HCMV virus propagation. It has been previously reported that Interferon type I (IFN-I) is able to induce APOBEC3 expression. In agreement with these results, we demonstrated that the induction of APOBEC3G expression upon HCMV infection is IFN-I mediated, produced early during HCMV infection. Therefore, understanding the role of this deaminase on the immune response against HCMV has become pivotal.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-031

The HCMV US27 gene product targets transcription factor NRF-1 to upregulate CXCR4

Juliet Spencer¹, Jordan Boeck¹, Christine O'Connor²

¹University of San Francisco, Biology, SAN FRANCISCO, United States of America

²Cleveland Clinic, Genomic Medicine Institute, CLEVELAND, United States of America

Human cytomegalovirus (HCMV) establishes lifelong latent infection in myeloid progenitor cells residing in the bone marrow, yet how virus enters the bone marrow to establish this latent reservoir remains unknown. The CXCR4/CXCL12 signaling pathway plays a central role in hematopoiesis and trafficking of cells to and from the bone marrow. HCMV encodes four G-protein coupled receptor (GPCR) homologs, US27, US28, UL33, and UL78, and three of these have been found to influence CXCR4 signaling activity when expressed individually in transfected cells. UL33 and UL78 both impair CXCR4/CXCL12 signaling, while US27 has been found to potentiate CXCR4/CXCL12 signaling, resulting in greater calcium flux and cell migration. In order to investigate the effect of US27 on CXCR4 during virus infection with other viral GPCRs present, fibroblasts were infected with HCMV strain TB40wt or mutants lacking US27 (TB40/deltaUS27), all four GPCRs (TB40/ALLDEL), or a mutant expressing only US27 and not the other three GPCRs (TB40/US27 *wt*). CXCR4 gene expression was significantly higher in TB40wt and TB40/US27 *wt*-infected fibroblasts compared to controls, and this effect was seen as early as three hours post infection, suggesting that US27 from the virus particle could enhance CXCR4 expression. Reporter gene assays confirmed that US27 increased transcriptional activity mediated by NRF-1, the primary transcription factor for CXCR4. Treatment with wortmannin or LY249002 ablated the increase in NRF-1 activity, indicating that phosphatidylinositol-3 kinase (PI3K) plays a role in the US27-mediated increase in CXCR4 expression. Immunofluorescence microscopy revealed increased translocation of NRF-1 into the nucleus of TB40wt-infected cells compared to mock or TB40/deltaUS27-infected cells. These results demonstrate that US27 increases CXCR4 gene expression by influencing activity of transcription factor NRF-1 in a PI3K-dependent manner. This work may reveal a novel regulatory function for an orphan receptor that could have implications for the establishment or maintenance of HCMV latency.

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Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-032

Genomic analysis of chimeric human cytomegalovirus vaccine candidates derived from strains Towne and Toledo

Andrew Davison¹, Nicolás Suárez¹, Betty Lau¹, George Kemble², Ronzo Lee³, Edward Mocarski⁴, Gavin Wilkinson⁵, Stuart Adler³, Michael McVoy³

¹MRC-University of Glasgow Centre for Virus Research, GLASGOW, United Kingdom

²3-V Biosciences, Inc., MENLO PARK, CA, United States of America

³Virginia Commonwealth University, RICHMOND, VA, United States of America

⁴Emory University, ATLANTA, GA, United States of America

⁵Cardiff University, CARDIFF, United Kingdom

Human cytomegalovirus (HCMV) is an important opportunistic pathogen in immunocompromised patients and a major cause of congenital birth defects when acquired *in utero*. In the 1990s, four chimeric viruses were constructed by replacing genome segments of the high passage Towne strain

with segments of the low passage Toledo strain, with the goal of obtaining live attenuated vaccine candidates that remained safe but were more immunogenic than the overly attenuated Towne vaccine. The chimeras were found to be safe when administered to HCMV-seronegative human volunteers, but to differ significantly in their ability to induce seroconversion. This suggests that chimera-specific genetic differences impacted the ability to replicate or persist *in vivo* and the consequent ability to induce an antibody response. To identify specific genomic breakpoints between Towne and Toledo sequences and establish whether spontaneous mutations or rearrangements had occurred during construction of the chimeras, complete genome sequences were determined. No major deletions or rearrangements were observed, although a number of unanticipated mutations were identified. However, no clear association emerged between the genetic content of the chimeras and the reported levels of vaccine-induced HCMV-specific humoral or cellular immune responses, suggesting that multiple genetic determinants are likely to impact immunogenicity. In addition to revealing the genome organization of the four vaccine candidates, this study provided an opportunity to probe the genetics of HCMV attenuation in humans. The results may be valuable in the future design of safe live or replication-defective vaccines that optimize immunogenicity and efficacy.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-033

Next Generation Sequencing of CMV Genomes from Congenitally Infected Infants Identifies Variants Associated with Newborn disease and CMV-related Hearing Loss

G. Clement Dobbins¹, Amit Patki², Curtis Hendrickson², William J. Britt², Karen Fowler², Suresh B. Boppana², Shannon A. Ross²

¹The University of Alabama at Birmingham, Department of Pediatrics, BIRMINGHAM, United States of America

²University of Alabama at Birmingham, Biostatistics, BIRMINGHAM, United States of America

Background: Congenital cytomegalovirus (cCMV) is the most common congenital infection in children and leads to permanent disabilities including sensorineural hearing loss (SNHL). Clinical or laboratory markers for identifying infected children at increased risk for sequelae are not known.

Objective: To identify CMV variants associated with symptomatic infection and SNHL utilizing whole genome sequencing of specimens from infected infants.

Methods: CMV from urine specimens of 33 infected infants were deep sequenced (Illumina MiSeq) after CMV target enrichment via custom capture probes. CLC Genomics Workbench software version 9.0 was used for assembly and analysis of sequences. Variants were compared between symptomatic and asymptomatic infants and between children with and without hearing loss. Statistical significance of cohort variant frequency distribution was determined using Fisher exact test.

Results: Sequencing achieved >95% coverage of the genome; and 7.6×10^7 reads were successfully mapped to the reference strain Merlin. Over 400 variants were found to be associated with symptomatic infants. Most non-synonymous variants (NSVs) associated with symptomatic cCMV were found in the known antigenic domains of UL55 (gB). Six of these variants were in close proximity to residues that when mutated could reduce antibody binding. When sequences from children with and without hearing loss were compared, 313 NSVs were associated with SNHL and the majority were in coding regions UL8 and RL8.

Conclusions: CMV genomes in urine specimens from infants with cCMV can be sequenced with >95% coverage and >10-fold depth. Several NSVs in the antigenic domains of gB were associated with symptomatic infection suggesting a role for virus diversity in newborn disease. In addition, unique NSVs within the coding regions, UL8 and RL8 were seen in children with SNHL. These findings highlight the need for additional studies to define the role of these polymorphisms in the pathogenesis and as markers of disease and outcome.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-035

The constitutively active HCMV-encoded G protein-coupled receptor UL33 displays oncomodulatory potential

Jeffrey van Senten¹, Tian Shu Fan¹, Raimond Heukers¹, Tonny Lagerweij², Thomas Würdinger², Marco Siderius¹, Martine Smit¹

¹Vrije Universiteit Amsterdam, Medicinal Chemistry, AMSTERDAM, Netherlands

²VU Medical Center, Cancer Center Amsterdam, Neuro-Oncology Research group, AMSTERDAM, Netherlands

The human cytomegalovirus (HCMV) encodes the constitutively active G protein-coupled receptors (GPCRs) US28 and UL33. Both receptors show high homology to human chemokine receptors and multiple chemokines have been identified to bind US28. For UL33 no endogenous ligands have been reported to date. US28 possesses oncomodulatory properties and is detected in HCMV positive human glioblastomas.

In this study we show that UL33 constitutively activates various pro-inflammatory, proliferative and pro-angiogenic signal transduction pathways when expressed in HEK293T and U251 malignant glioblastoma cells. Moreover, expression of UL33 in glioblastoma cells increased spheroid size *in vitro* and promoted tumor growth in an orthotopic xenograft mouse model. Altogether, our data indicate that the viral GPCR UL33 has oncomodulatory potential and, together with US28, could play a role in HCMV-associated malignancies.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-036

About the impact of reinfection and persistence in host-pathogen systems subject to balancing selection

Cornelia Pokalyuk

Goethe University - Institute for Mathematics, FRANKFURT, Germany

For many open reading frames (ORF) of the CMV genome, published DNA sequence samples cluster around a few, well-distinguishable genotypes. Sequences lying in between these genotypes are not observed, suggesting they are strongly deleterious or even not viable. This fitness valley between genotypes makes the occurrence of a genotype by spontaneous mutation a rare event, and therefore for most ORFs only a single genotype should exist in the viral population. Given the opposite observation the question arises whether genotypes are actively maintained by balancing selection in the CMV population. We will deal with this scenario for host-pathogen systems, in which the pathogens are capable of persistence and reinfection, and make predictions about the genotype frequencies which should be observed in such populations of pathogens. Furthermore, we will discuss the impact of reinfection and persistence for pathogen survival in this context.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-037

Induction of chemokines and chemokine receptors of glioblastoma infected with human cytomegalovirus

Masaya Takemoto

Hokuriku University, Faculty of pharmaceutical sciences, KANAZAWA, Japan

Glioblastoma (GBM) is the most malignant brain tumor that accounts for approximately 37% of gliomas with a 5-year survival rate of 6.9% and an average survival time of 12 months. In recent years, transcripts and proteins of human cytomegalovirus (HCMV) have been successively reported to be detected in GBM patients. We found and reported so far that HCMV infection increases the expression of host chemokines and chemokine receptors, e.g. IL-8, MCP-1, I-TAC, RANTES, CXCR1, and CCR2. Although these proinflammatory chemokines and their receptors are known to be involved in the pathogenesis of GBM, it remains to be proved that they are upregulated in GBM with HCMV infection. Thus, we examined the induction of chemokines and chemokine receptors in GBM cell lines infected with HCMV.

Two GBM cell lines, T98G and A172, and an astrocytoma cell line, U373-MG, were infected with HCMV Towne and clinical strain, 91S. Thirteen kinds of chemokine and eleven kinds of cytokine were screened in the culture supernatant of the infected cells by using LEGENDplex immunoassay (BioLegends) and several chemokines including MCP-1 and IL-8 were found to be elevated with infection of both or either Towne or 91S. The expression level of the receptors, CCR2A, CCR2B, CXCR1, and CXCR2 was analyzed by real-time RT-PCR, and it was demonstrated that either CCR2A or CCR2B was also upregulated while neither CXCR1 nor CXCR2 was changed. Furthermore, we addressed whether HCMV infection may induce the same effect in the glioma stem-like cells (GSLCs) that were induced from the three cell lines by culture in the stem cell medium. As a result, it was shown that HCMV-infected GSLCs also expressed increased level of IL-8, MCP-1, CCR2A, and CCR2B.

These results suggest that HCMV may play some roles in the pathogenesis of GBM via chemokine signaling such as MCP-1/CCR2 pathway.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-039

United we stand, divided we falter: mutual dependence of HCMV terminase subunits pUL51, pUL56, and pUL89 for terminase complex formation

Eva Maria Borst¹, Sebastian Neuber¹, Karen Wagner¹, Thomas Goldner², Peter Lischka², Martin Messerle¹

¹Hannover Medical School, Virology, HANNOVER, Germany

²AiCuris Anti-infective Cures GmbH, WUPPERTAL, Germany

Packaging of HCMV genomes into preformed capsids is an essential, virus-specific process that emerges as an auspicious drug target for novel antiviral strategies. One promising substance targeting the HCMV terminase, Letemovir, recently met the primary endpoint in a phase III clinical study, yet its precise mode of action is unknown. Thus, more knowledge about terminase formation and function is required to understand the mechanism of action of such new antiviral drugs.

To gain more insight into HCMV terminase assembly, we constructed HCMV BAC genomes in which the ORFs encoding either terminase subunit were disrupted. These studies were complemented by transient expression of the HCMV terminase proteins in different combinations. We found that in the absence of one terminase constituent the remaining ones did no longer efficiently interact with each other. Moreover, each subunit was required to maintain appropriate protein levels of the other two. Subsequent analyses revealed that when one terminase subunit was missing, the others underwent proteasomal degradation. Adding the respective missing subunit in *trans* rescued the protein levels. Furthermore, nuclear localization of pUL51 and pUL89 occurred only when all three terminase components were expressed together.

Our findings point to a model of the HCMV terminase as a tripartite multi-protein complex in which the individual subunits stabilize each other upon complex formation. Such a scenario is reminiscent of the

folding-upon-binding principle leading to cooperative stability in large protein assemblies. These findings on the mutual interplay of the HCMV terminase subunits will be useful for investigating the molecular mechanisms underlying inhibition by new antiviral drugs that target HCMV genome encapsidation.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-040

Human Cytomegalovirus Infected Colorectal Cancer Cell Lines Increase the Cell Proliferation and Migration Activities

Yu Jiun Chan¹, Wan Huai Teo², Hsin Pai Chen³

¹Taipei Veterans General Hospital, TAIPEI, Taiwan

²NATIONAL YANG MING UNIVERSITY, TAIPEI, Taiwan

³TAIPEI VETERANS GENERAL HOSPITAL, TAIPEI, Taiwan

Background: Recent studies had demonstrated that human cytomegalovirus (HCMV) could be found in the colorectal cancer (CRC) tumorous cells and affect outcome, indicating its potential onco-modulator role. This study further investigated the effects of HCMV infection in CRC cell lines and looked for the phenotypic changes.

Material/methods: The laboratory strain, HCMV AD169, was used to infect the colorectal cell line (HT29 cell, for 24, 48, and 72 hours) and its derived "spheroid" cells that were pre-treated by the *in vitro* tumoursphere formation assay to enhance the viral infectivity rate. Cell proliferation was evaluated by a colorimetric assay to determine cellular viability by measuring the metabolic conversion of a water-soluble tetrazolium salt (WST-1). The cell viability was carried out by direct cell count. The cell migration ability was determined by using transwell migration assays. The expression levels of epithelial-mesenchymal transition (EMT)-related genes were examined by using RT² Profiler PCR Array (SABiosciences, USA).

Results: After 24, 48 and 72 hours post-infection of HCMV either in HT29 cells or HT29 "spheroid" cells, the cell proliferation activities significantly ($P < 0.0001$) enhanced. Infection of HCMV also significantly ($P < 0.0001$) enhanced the cell viability. In addition, infection of HCMV in HT29 cells and HT29 "spheroid" cells also promoted the cell migration. The migration rate in AD169 infected HT29 "spheroid" cell is 3 fold higher compared to AD169 infected HT29 cells. Using EMT assays, 21 genes were identified with differentially expression (cut off value >2) between infected and non-infected cells. One of the most significant changes was the Wingless-type MMTV integration site family (Wnt) signaling.

Conclusions: HCMV infected CRC cell lines resulted in a significant phenotypic alterations in proliferation and migration activities. Meanwhile, The Wnt signaling pathway was activated in the presence of HCMV infection.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-043

Characterisation of Human Cytomegalovirus US12 gene family-encoded proteins

Hester Nichols¹, Ceri Fielding¹, Peter Tomasec¹, Michael Weekes², Luis Nobre², Paul Lehner², Andrew Davison³, Gavin Wilkinson¹

¹Cardiff University, Infection and Immunity, CARDIFF, United Kingdom

²Cambridge Institute for Medical Research (CIMR), CAMBRIDGE, United Kingdom

³University of Glasgow Centre for Virus Research, GLASGOW, United Kingdom

The Human Cytomegalovirus (HCMV) genome contains a remarkable array of 13 gene families ranging in size from 2 to 14 members. The US12 gene family consists of a sequential tandem array of 10 genes designated US12 to US21 that are highly conserved in clinical isolates. Two US12 family members, US18 and US20, act in concert to target the natural killer (NK) cell activating ligand MICA for lysosomal degradation (Fielding, CA *et al.*, 2014. PLoS Pathogens 10 (5):e1004058). Fibroblasts infected with a library of US12 gene family deletion mutants were used in NK degranulation assays and cell surface proteomics. This analysis revealed that 2 additional US12 family members contribute to NK cell evasion and that the US12 family down-regulate an impressive array of immune ligands at the cell surface. To complement these mechanistic studies into US12 family function, we added a C-terminal V5 epitope tag to each individual US12 family gene ORF within the HCMV Merlin genome and successfully generated HCMV virus stocks. Expression of US12 family members was detectable by immunoblotting, with a subset identified as N-glycosylated proteins. This glycosylation could have important implications for their function. Furthermore, the majority of US12 family members were targeted for lysosomal degradation and we are currently investigating the hypothesis that US12 family proteins may directly interact with their targets to re-direct them to the lysosome for degradation.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-045

Influence of maternal hyperimmune globulin (HIG) infusion on viral diversity and congenital transmission

Diana Vera Cruz¹, Cody S Nelson², Kristy M Bialas², Dollnovan Tran³, Peter A Barry⁴, Amitinder Kaur³, Katharina Koelle¹, Sallie R Permar²

¹Duke University, Biology, DURHAM, United States of America

²Duke University Medical Center, Human Vaccine Institute, DURHAM, United States of America

³Tulane University, Tulane National Primate Research Center, COVINGTON, United States of America

⁴University of California, Davis, Center for Comparative Medicine, Depart, DAVIS, United States of America

Congenital CMV infection is the leading infectious cause of birth defects worldwide, and interventions to prevent this transmission are needed. In this work, we investigate how preexisting anti-CMV antibodies shape the maternal viral population and their effects on intrauterine transmission. We analyzed genomic data from a newly-defined rhesus monkey model of congenital rhesus CMV (RhCMV) infection to examine the effects of pre-infection, passively-infused hyperimmune globulin (HIG) on viral population genetics in both maternal and fetal compartments. Three different strains of RhCMV were simultaneously inoculated intravenously into CD4+ T cell-depleted rhesus monkeys following prior treatment with either a standard HIG preparation or a high-neutralizing potency preparation. Samples were collected weekly after infection and utilized for targeted amplicon deep sequencing of the glycoprotein L (gL) and B (gB) genes. The epithelial cell-tropic strain UCD52 constitutes the majority of the population regardless of treatment or tissue type. Maternal plasma viral populations from the high-potency group were less diverse (mean $p=0.98e-3$ per site) than those from control ($1.38e-3$ per site) or standard HIG groups ($1.85e-3$ per site). Further, amniotic fluid populations were more diverse in the standard ($1.85e-3$ per site) than in the control group ($0.80e-3$ per site). Interestingly, the highest levels of genetic diversity were observed in amniotic fluid in the first weeks after transmission within the standard group ($2.58e-3$ per site). In one dam-fetal pair, analysis of diverse RhCMV positive fetal tissues revealed a high fraction (~75%) of shared, genetically similar haplotypes. More haplotypes were shared between fetal tissues, including amniotic fluid, than between populations from different maternal tissues, suggesting a higher degree of compartmentalization within the mother than the fetus. Our analysis indicates that pre-existing antibodies may select for certain CMV haplotypes, highlighting the relevance of analyzing viral evolutionary dynamics in the study of clinical strategies to impede congenital CMV transmission.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-046

Cytomegalovirus downregulates ER- α , PR and Her-2 Receptors in Breast Cancer -A potential role in triple negative breast cancer?

Helena Costa¹, Abdul-Aleem Mohammad¹, Afsar Rahbar¹, Joel Touma², Belgis Davoudi¹, Ida Rashid Bukholm², Torill Sauer², Katja Vetvik², Jürgen Geisler², Cecilia Söderberg-Naucler¹

¹Karolinska Institutet, STOCKHOLM, Sweden

²Akershus University Hospital, Department of Breast and Endocrine Surg, LØRENSKOG, Norway

Breast cancer (BC) is the fifth most common cause of cancer deaths. The most important prognostic markers for BC are the hormone receptors Estrogen receptor alpha (ER- α) and progesterone receptor (PR). Anti-hormone therapy has increased the survival of patients with cancers expressing these two hormone receptors. A worse prognosis is associated with amplification of Her-2 (Her-2 positive) or absence of these three receptors (triple negative breast cancer, TNBC). While Her-2 positive breast cancer patients can be offered Her-2 directed therapy with improved patient outcome, TNBC patients currently lack effective therapies. Our group and others have shown high prevalence of Human Cytomegalovirus (HCMV) in primary BC and in lymph nodes and brain metastases of breast cancer patients. Patients with higher-grade of HCMV protein expression had worse prognosis, with shorter time to progression and shorter survival. To assess a potential correlation between HCMV and the expression of ER, PR and Her-2, we graded primary breast tumors (n=62) for HCMV protein expression and correlated the results with clinical data of ER- α , PR and Her-2 status. High-grade HCMV expression correlated inversely with ER- α ($p = 0.02$) and PR ($p = 0.003$) expression. Her-2 expression also trended to be reduced ($P = 0.09$) in HCMV-positive samples. *In vitro*, HCMV infection resulted in significant downregulation of mRNA and protein levels of ER- α /PR and Her-2 in BC cell lines, an effect that was dependent on viral gene expression. Three HCMV encoded miRNAs that could target ER- α /PR expression were identified by bioinformatics analysis: miR-UL22A-3p, miRUL36-3p and miR-US25-2-3p. Overexpression of each viral miRNAs in breast cancer cells resulted in downregulation of mRNA and protein levels of ER- α /PR. Thus, HCMV may promote establishment of TNBC, through viral miRNA induced downregulation of ER- α /PR and Her-2. Antiviral therapy could hence be a potential alternative treatment strategy for selected breast cancer patients.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-047

RL13 and the UL128 Locus co-operate in promoting direct cell-to-cell transmission of human cytomegalovirus.

Carmen Bedford¹, Robin Antrobus², Michael Weekes², Paul Lehner², Gavin Wilkinson¹, Richard Stanton¹

¹Institute of Infection and Immunity, School of Medicine, Cardiff University, CARDIFF, United Kingdom

²Cambridge Institute for Medical Research, CAMBRIDGE, United Kingdom

Propagation *in vitro* of CMV strains truly representative of clinical virus is urgently required to facilitate the development of antivirals, immunotherapies and vaccines. However, experience shows that efficient productive replication of clinical CMV strains in cell culture is generally achieved only after two independent mutations have been selected for. These mutations incapacitate the UL128 locus (UL128L) and the hypervariable gene RL13. The UL128L encodes three components of a pentameric complex present in the virion envelope, and is implicated in tropism. RL13 encodes a hypervariable virion envelope protein that suppresses release of infectious virions from cells. In this study, we show that

despite limiting virus release, RL13 does not impair the temporal cascade of CMV gene expression during productive infection of fibroblasts. Nor were there any effects on intra-cellular particle maturation by electron microscopy. However RL13 expression was associated with both a reduction in the numbers and 'infectivity' of particles released from cells, indicating that its expression affects virus egress. We demonstrate that both RL13 and UL128L independently promoted direct cell-to-cell spread that was resistant to neutralising antibodies in fibroblasts. Yet RL13 expression in epithelial cells promoted virus that was more sensitive to neutralising antibodies, indicating that RL13 affects the mechanism of cell-cell spread in a cell-type specific manner. A SILAC-IP identified cellular proteins that were recruited to the virus assembly compartment by RL13 during productive infection. Using siRNA knockdown of these proteins we hope to elucidate the protein-RL13 interactions that are relevant to RL13 function.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-050

Viral Effective Population Size and Selection Coefficient Reveal Disease Status and Treatment Outcome

Laura Gibson¹, Nicholas Renzette¹, Mark Schleiss², Marisa Mussi-Pinhata³, Aparecida Yamamoto³, Michael Keller⁴, Catherine Bollard⁴, Sophia Alain⁵, William Britt⁶, Jeffrey Jensen⁷, Timothy Kowalik¹

¹University of Massachusetts Medical School, Departments of Medicine and Pediatrics, WORCESTER, MA, United States of America

²University of Minnesota Medical School, MINNEAPOLIS, MN, United States of America

³University of São Paulo, SÃO PAULO, Brazil

⁴Childrens National Health System, WASHINGTON, DC, United States of America

⁵Faculté de Médecine-Université de Limoges, LIMOGES, France

⁶University of Alabama Birmingham School of Medicine, BIRMINGHAM, AL, United States of America

⁷Ecole Polytechnique Fédérale de Lausanne (EPFL), LAUSANNE, Switzerland

BACKGROUND Within individuals, HCMV populations maintain stable structures based on tissue compartments and patterns of evolution over time. However, structures may destabilize during antiviral therapeutic interventions.

METHODS Longitudinal blood and urine samples were obtained from 16 immunocompromised patients with different treatment approaches (none, antiviral drugs, immunotherapy). HCMV populations were analyzed to identify parameters (census size, effective population size, selection coefficient) that correlate with disease status (symptomatic, asymptomatic) or treatment outcome (DNAemia resolution or rebound).

RESULTS While census size (number of individuals in a population, e.g. "viral load") did not correlate with disease status or treatment outcome, estimates of effective population size or selection coefficient resulted in patterns that distinguish these clinical features.

Effective population size (N_e) is the number of individuals generating progeny. Patients with asymptomatic untreated HCMV infection had large N_e values and stable population structures over time. In contrast, symptomatic patients with resolution of DNAemia after treatment had low N_e values, consistent with the theory that low N_e leads to extinction. However, patients with symptomatic untreated infection or with treatment failure had mid-range N_e values.

Selection coefficient (s) measures fitness by comparing growth rate of mutant versus wild type (WT) virus. WT sequences have $s=0$, and negative or positive values represent polymorphisms in mutant sequences with reduced or enhanced fitness, respectively. Polymorphisms in patients with untreated infection had WT fitness profiles ($s=0$). In contrast, those in patients with treatment failure showed enhanced fitness ($s>0$). These polymorphisms were not associated with resistance to antiviral drugs or escape from HCMV-specific adoptive T cells, but were found in ORFs with unclear functions or encoding proteins not used to generate T cells.

CONCLUSIONS In immunocompromised patients, disease status and treatment outcome segregate by effective population size, and positive selection targets treatment-unrelated ORFs to provide fitness advantage in rebound viral populations.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-054

Variability of gB UL55 from whole genome sequencing of congenital isolates

Sophie Alain¹, Florence Agbazahou², Lilian Roland², Valentin Tilloy³, Emilie Guerin³, Sebastien Hantz¹

¹National Reference Center for Cytomegaloviruses, CHU Limoges, Virology, LIMOGES, France

²Inserm U 1092, University of Limoges, National Reference center for CMV, LIMOGES, France

³GenoLim/Biscem genomic platform, University of Limoges, National Reference center for CMV, LIMOGES, France

Human cytomegalovirus is the first cause of birth defect though viral determinants for neurological damages are not well established. High throughput sequencing, enables the analysis of the whole HCMV genome with a high precision. We thus compared the whole genome sequence of 5 low passage (1 to 2 passages) clinical isolates from congenitally infected newborns with or without symptoms to the Merlin (ATCC) and AD169 genomes. Isolate F came from a child which developed unilateral hearing loss, S from an asymptomatic child, C, L and B from children with severe neurological symptoms. The whole intracellular HCMV genomes were isolated from infected human embryonic fibroblasts by the Hirt method, and libraries were directly prepared after human DNA depletion or not, on an ABI Library Builder, then sequenced by the Ion Torrent technology on a Ion Proton analyzer (Life Technologies). Bioinformatic analysis was performed on the GenoLim platform of Limoges University using Mira software after subtraction of human DNA (47%, with or without depletion). The mean reads number per base was 17091 with a 1% error risk and a coverage of the whole genome. Within a panel of 24 genes implicated in tropism permissivity and temperance, the highest variability was observed in UL9, UL10, UL55 and UL75. Compared to Merlin-ATCC and AD169, we identified two highly variable adjacent regions within AD2 and CLS domains which differed between isolates from symptomatic and mild/asymptomatic infections, and one highly conserved region between AD4 and AD5 antigenic domains. In conclusion, further analysis of a larger panel of isolates is necessary to confirm the statistical significance of intra-AD2 region variants. Control by *de novo* analysis is ongoing. This Hirt-NGS method is easy to use and can provide wide range analysis of the full length HCMV genome with no need for capture or human DNA depletion.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-055

Guinea Pig CMV pp65 or pp71 Null Mutants have Opposite Phenotypes to Each Other in Tissue Culture and in Animals

K. Yeon Choi, Matthew Markert, Alistair McGregor

Texas A&M University Health Science Center, COLLEGE STATION, United States of America

The guinea pig is the only small animal model for congenital CMV (cCMV) and important in studies related to disease and intervention strategies. The animal genome was recently sequenced which enabled an in-depth evaluation of cellular gene expression in response to infection. Potentially, this approach may provide a better understanding of infection at the molecular level in relation to viral invasion of the placenta. As a premise to cCMV infection studies, wild type (WT) and mutant guinea pig CMV (GPCMV) infection was evaluated in primary embryo fibroblast (GEF) cells by cellular transcriptome analysis. Individual deletion mutants of genes encoding the homolog pp65 (GP83Km) or pp71 (GP82Km) were generated. Both mutants had increased susceptibility to type I interferon but

GP83Km had near normal growth, whereas the GP82 mutant had delayed kinetics. In animal pathogenicity studies, GP82Km dissemination was similar to WT but GP83Km was highly attenuated. The cellular transcriptome expression pattern of mock, WT, GP82Km and GP83Km mutant virus infected GEFs (moi=1pfu/cell) was evaluated (1-72 hpi). Transcriptome analysis was performed using a chip microarray with over 25,500 genes evaluated for each time point. Genes that were upregulated or downregulated more than two-fold compared to mock were identified. Analysis revealed a complex and divergent pathway of cellular gene modification in response to infection. Over 3,300 genes were differentially regulated by each virus over the studied period and approximately 21% were immune related. Overall, WT and GP83Km had a more similar pattern of gene dysregulation but became more divergent at later stages of infection. GP82Km had a more distinct pattern of gene regulation with chemokines (CCL2, CCL5, CXCL11, IL8) and pro-inflammatory factors (Fos, IL6, Fosb, Tnfaip3, Tnfsf8, Atf3) upregulated to much higher levels(>10 fold over WT) during early stages of infection which resulted in upregulation of additional downstream targets at later stages.

380 - Poster Presentation Track B

Date: 02/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

B-PP-056

Evaluation of guinea pig CMV (GPCMV) IE1 and IE2 as Functional HCMV Homologs and Potential Vaccine T cell target antigens

Alistair McGregor, Julia Hornig, K. Yeon Choi

Texas A&M University Health Science Center, Microbial Pathogenesis & Immunology, COLLEGE STATION, United States of America

The guinea pig is the only small animal model for congenital CMV and therefore important for the development of CMV intervention strategies. In HCMV, IE1 and IE2 proteins are required for the viral lytic life cycle and in part necessary to overcome innate immunity. Additionally, IE1 is considered an important T cell target for protective immunity. We characterized GPCMV IE1 and IE2 to demonstrate functional homology to HCMV. GPCMV major IE genes are splice variants with three common shared exons and a unique fourth exon: *GP122* (IE2); *GP123* (IE1). Transient expression of GPCMV IE1 and IE2 demonstrated nuclear colocalization with each other, the viral polymerase and guinea pig ND10 components (gpPML, gpDaxx, gpSp100, gpATRX). N- and C-terminus deletion variants of IE1 mapped the nuclear localization signal to the C-terminus. Separate knockout of GPCMV *GP122* and *GP123* were lethal which demonstrated their essential nature. In contrast, a MCMV *M123* knockout was viable. A truncated *GP123* mutant virus (vIET) resulted in a viable virus (IE1 codons 233-474 deleted). vIET had increased susceptibility to type I IFN (95% inhibition at 100U/ml) compared to a pp65 homolog mutant and wildtype virus (80 and 5% inhibition respectively). The truncated IE1 protein was unable to interact with gpPML compared to full length IE1 or HSV ICP0. Cellular transcriptome studies demonstrated a different phenotype for vIET compared to wildtype or pp65 knockout virus. In tissue culture, vIET had impaired kinetics compared to a pp65 mutant but in animals a pp65 knockout virus was more impaired. However, vIET had decreased titers for all organs (lung, liver, spleen, and salivary glands) at all time points (4, 8, 12, and 27 dpi) compared to wildtype virus. An IFN γ ELISPOT assay demonstrated that IE1 was a T cell target in infected animals which suggested it could be useful for vaccine studies.

400 - Session C.03: Clinical Practice, Problems & Solutions

Date: 03/05/2017

Time: 08:30 - 10:25 hrs

Invited presentation

C-INV-003

An economic view on T cells: Inflation and deflation

Annette Oxenius, Nicolas Baumann, Nicole Torti

CD8 T cells play a crucial role for the control of lytic murine CMV (MCMV) replication and for the surveillance of reactivation events during viral latency. Certain MCMV-specific CD8 T cells undergo an unconventional response kinetics, so called memory inflation. 'Inflationary T cells' expand during acute virus replication and continue to proliferate and expand even after control of lytic replication. They accumulate to high numbers in peripheral organs where the population is maintained at constant levels and exhibits an effector-memory phenotype - a characteristic that is currently harnessed in vaccine approaches.

We elucidated the mechanisms promoting stable maintenance of inflationary MCMV-specific CD8 T cells in peripheral tissues. We found that antigen presentation on non-hematopoietic cells is required for memory inflation in a continuous process. Blocking continuous seeding of cells to peripheral organs revealed an intrinsic half-life of this population in the range 40-60 days, indicating that the overall stability of the population required replenishment of 50% of the cells every 6 to 8 weeks. This continuous replenishment is likely fueled by egress of reactivated MCMV-specific CD8 T cell from lymph nodes, as we found that latently infected non-hematopoietic cells in lymph nodes reactivate MCMV-specific central memory CD8 T cells, leading to their proliferation, differentiation into effector cells, and migration to peripheral tissues.

We further show that the intrinsic population half-life of 40-60 days of inflationary cells in peripheral organs is comparable in naïve and latently MCMV infected mice, arguing against a role of cognate antigen regulating the longevity of the population. Instead, we found a critical role for tissue IL-15 in promoting survival of the effector memory MCMV-specific CD8 T cell pool in the lung. We are currently identifying the cellular source of IL-15 in the lung as one representative peripheral organs that accommodates large numbers of inflationary cells.

400 - Session C.03: Clinical Practice, Problems & Solutions

Date: 03/05/2017

Time: 08:30 - 10:25 hrs

C-KEY-001

Adoptive T cell therapy of CMV infection

Dirk Busch

Technische Universität München, Institut für Med. Mikrobiologie, Immunologie und Hygiene, MÜNCHEN, Germany

Immunocompromised patients, e.g. upon allogeneic hematopoietic stem cell transplantation (alloHSCT), are of high risk to develop life-threatening infections by pathogens that are well controlled in healthy individuals, including CMV. CD8⁺ T cells play a pivotal role in providing protection against infections with intracellular pathogens (and some tumors) and therefore, the development of adoptive cell therapies (ACT) using antigen-specific T cells is currently gaining substantial clinical interest. Recent data indicate that most effective ACT requires *in vivo* expansion as well as long-term maintenance of transferred cells. Although it becomes evident that clinical pre-enrichment for defined T cell subsets might help to make engraftment and maintenance of T cells upon ACT more predictable, it is still controversially discussed which subset to choose. Over the past years we have developed single-cell adoptive transfer technologies combined with reversible cell labeling (so-called Streptamer technology) and polychromatic multiparameter flow cytometry of rare event populations. This novel type of single cell fate mapping has allowed us to demonstrate unequivocally that individual precursor cells within the naive and memory T cell pool can bear the full plasticity to develop into a plethora different T cell subsets. These observations have important implications for ACT since they demonstrate how it is possible to reconstitute effector and memory populations from very low numbers of adoptively transferred T cells. We are currently exploring Streptamer-based cell purification platforms to enable clinical purification of most effective T cell populations for ACT. A first clinical trial using small numbers of primary Streptamer-enriched CMV-specific T cells, without any *in vitro* culture, for treatment of therapy-refractory CMV disease in patients upon alloHSCT has just been completed and the overall promising results will be presented. New concepts for prophylactic treatment of infections in patients upon alloHSCT evolved from these observations and are currently tested in ongoing clinical trials.

400 - Session C.03: Clinical Practice, Problems & Solutions

Date: 03/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

C-OP-004

Analysis of T cells targeting human cytomegalovirus by Next Generation Sequencing

Alina Huth¹, Xiaoling Liang¹, Stefan Krebs², Helmut Blum², Andreas Moosmann¹

¹Helmholtz Zentrum München, AGV, MUNICH, Germany

²Genzentrum der Ludwig-Maximilians-Universität München, LAFUGA, MUNICH, Germany

T cells play a crucial role in control of HCMV latency and reactivation. Owing to somatic recombination and pairing of distinct α - and β -chains to form a functioning T cell receptor (TCR), the human T cell repertoire is greatly diverse. HCMV is a large pathogen with various immunogenic proteins. Depending on the HLA type of an individual, different viral epitopes are presented leading to activation of different sets of HCMV-specific T cells. Hence, the overall T cell response against HCMV is complex and challenging to analyse.

To tackle this task, we enriched virus-specific T cells by *in vitro* stimulation with peptide epitopes, peptide pools, or transformed B cells presenting epitopes from a full-length HCMV antigen. From these T cells, we isolated mRNA and prepared libraries for Next Generation Sequencing. HCMV-specific clonotypes were identified by comparison of T cell samples before and after stimulation.

We checked TCRs responding to externally loaded peptide against those responding to cells presenting endogenously processed epitopes. Our data show that the most frequent HCMV-specific TCRs detected by single peptide stimulation also recognise naturally presented epitopes. Likewise, we determined the portion of TCRs specific for currently unknown epitopes to evaluate the immunogenicity of our tested epitopes. We further identified immunodominant virus-specific TCRs, which were exclusive to HCMV seropositive donors. Intriguingly, these TCRs were already abundant in the unstimulated *ex vivo* repertoire, indicating that HCMV strongly shapes the T cell repertoire of its host.

Moreover, we found highly similar families of TCRs targeting HCMV epitopes in the majority of virus carriers. Such public TCR families have potential for the development of engineered T cells for adoptive transfer, vaccines, or novel HCMV disease monitoring strategies. Besides, identification of structural TCR motifs may enable to predict whether a TCR is specific for HCMV.

400 - Session C.03: Clinical Practice, Problems & Solutions

Date: 03/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

C-OP-005

Interaction between soluble HLA-G expression and HCMV infection during pregnancy

Tiziana Lazzarotto¹, Roberta Rizzo², Claudia Pavia¹, Liliana Gabrielli¹, Francesca Cervi³, Maria Grazia Capretti⁴, Daria Bortolotti², Valentina Gentili², Giuliana Simonazzi³, Giulia Piccirilli¹, Angela Chiereghin¹, Gabriele Turello¹, Diego Squarzoni¹, Dario Di Luca², Tiziana Lazzarotto¹

¹O.U. of Microbiology, Policlinico S. Orsola-Malpighi, University of Bologna, BOLOGNA, Italy

²Department of Medical Sciences, University of Ferrara, FERRARA, Italy

³O.U. of Obstetrics and Gynecology, University of Bologna, BOLOGNA, Italy

⁴O.U. of Neonatology, St. Orsola-Malpighi University Hospital, BOLOGNA, Italy

The fetal compartment can be studied by amniocentesis and ultrasound examination for the diagnosis and prognosis of HCMV infection. Since amniocentesis is an invasive procedure and positive results of amniotic fluid (AF) tests do not discriminate between infected fetuses and compromised fetuses, researchers continue to work on the prognosis factors for the HCMV disease. In order to improve the identification of i) pregnant women who transmit the virus to their fetus and ii) HCMV-infected and

compromised fetuses, we studied the expression of soluble isoform of the human leukocyte antigen class I "non-classical" (sHLA-G) during HCMV infection in maternal blood and amniotic fluid samples. HLA-G antigen is a tolerogenic molecule and is modified by HCMV infection, with possible functional consequences in pregnancy immuno-regulation. We describe the analysis of a clinical prospective trial enrolling 420 pregnant women suspected, at routine HCMV testing, to have active HCMV infection. The sHLA-G levels were evaluated in the plasma samples of 139 pregnant women with primary HCMV infection, 85 with non-primary, 155 with past infection, and 41 HCMV-uninfected. We found that the mean levels of sHLA-G in pregnant women with primary infection were significantly higher in comparison with the other mentioned groups (33.1 ± 2.4 ng/ml vs. 7.2 ± 1.0 ng/ml, 8.8 ± 1.4 ng/ml, 6.9 ± 2.1 ng/ml, respectively, $p < 0.001$). When we analyzed the levels of sHLA-G in plasma samples from primarily infected pregnant women, considering transmitter and non-transmitter mothers, we found a statistical correlation ($p = 0.035$). We analyzed 67 AF samples collected during amniocentesis (20-21 weeks gestation) from pregnant women with primary HCMV infection. The mean levels of sHLA-G were significantly higher in samples from infected fetuses (60.09 ± 17.74 ng/ml) than in uninfected fetuses (29.13 ng/ml) ($p < 0.002$). Finally, the difference of the means sHLA-G levels between urine from HCMV infected and uninfected newborns ($p = 0.002$) was statistically significant.

400 - Session C.03: Clinical Practice, Problems & Solutions

Date: 03/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

C-OP-006

Neurodevelopmental outcome and hearing in early childhood of preterm infants with postnatal cytomegalovirus infection

Julia Gunkel, Marian Jongmans, Corine Koopman-Esseboom, Ingrid C. Van Haastert, Maria C.J. Eijssermans, Petronella C. Fieret- van Stam, Bert G.A. Van Zanten, Tom F.W. Wolfs, Linda S. De Vries, Joppe Nijman

University Medical Center Utrecht, Neonatology, UTRECHT, Netherlands

Objective: To assess whether preterm infants with postnatal cytomegalovirus (pCMV) infection develop neurological sequelae in early childhood. Methods: Infants with a gestational age <32 weeks were prospectively screened for CMV at term-equivalent age. Neurodevelopment was assessed using the Griffiths Mental Development Scales (GMDS) at 16 months corrected age (CA), the Bayley Scales of Infant and Toddler Development-III (BSITD-III) at 24-30 months CA, the Movement Assessment Battery for Children-II (MABC-II) and/or the Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III) and hearing assessment, at 5.5 years of age. Results: Three-hundred-and-fifty-six infants were tested at 16 months CA (49 CMV+; 307 CMV-). At 16 months CA, infected infants performed better on the GMDS locomotor scale ($p = 0.025$). There were no differences at 24-30 months CA on the BSITD-III or GMDS. At 5.5 years of age, infected children scored lower on the WPPSI-III but the mean scores were within the normal range, reaching significance only on verbal IQ ($p = 0.046$). Multiple regression analysis indicated no impact of CMV status but significant influence of maternal education and ethnicity. There were no significant differences between both groups on the MABC-II. None of the infected children developed perceptible hearing loss at 5.5 years of age. Conclusion: Cognitive- and motor function in preterm children with a pCMV infection was within normal range in infancy. In early childhood, there was no significant difference in mean total IQ between both groups, but mean verbal IQ was significantly lower in infected infants. None of the infected children developed perceptible hearing loss.

420 - Session B.05: Virus and Host

Date: 03/05/2017

Time: 10:55 - 13:05 hrs

Invited presentation

B-INV-005

Mononuclear phagocyte subset activation and functions during murine cytomegalovirus infection

Marc Dalod

CNRS, CIML, MARSEILLE, France

Mononuclear phagocytes encompass a variety of cell types specialized in different functions, including plasmacytoid dendritic cells which are major producers of type I and III interferons (IFN-I/III) during viral infections, classical DC (cDC) encompassing XCR1+ cDC excelling in the activation of CD8 T cells in part through crosspresentation and CD11b+ cDC which promote Th2 and Th17 responses, macrophages which mediate microbicidal and tissue repair functions, and classical monocytes which upon inflammation migrate into inflamed tissues to differentiate in inflammatory monocytes, monocyte-derived dendritic cells, monocyte-derived macrophages or myeloid derived suppressor cells. Depending on the physiopathological context, cMo-derived cells can be beneficial or detrimental. There are major discrepancies between published reports on the role of cMo during murine cytomegalovirus (MCMV) infection. This may be due to the use of distinct strains of mice or of virus, to the study of different organs, or to the confusion existing in the field regarding the identity and the plasticity of the different types of cMo-derived cells. More generally, the role of the different subsets of mononuclear phagocytes during MCMV infection is not clear. We are trying to address this issue by using a systems biology approach combining gene expression profiling to generate novel hypotheses, with morphological, phenotypical and functional studies to test these hypotheses in wild-type mice or mutant animals allowing in vivo tracking, depletion or gene inactivation of subsets of mononuclear phagocytes. These studies will generate novel knowledge on the functions of mononuclear phagocyte subsets and their molecular regulation during MCMV infection which could then be used to study other physiopathological conditions including additional viral infections or cancer, ultimately allowing to identify novel targets for manipulating immune responses to promote health over disease.

420 - Session B.05: Virus and Host

Date: 03/05/2017

Time: 10:55 - 13:05 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-017

Human cytomegalovirus infection enhances the expression of genes critical for neural cell migration in neural stem cells and fetal brain

Stéphane Chavanas¹, Maude Rolland¹, Jacqueline Aziza², Yann Sellier³, Bettina Bessières³, Hélène Martin¹, Charlotte Casper¹, Alexandra Benchoua⁴, Marc Peschanski⁴, Marianne Leruez-Ville³, Daniel Gonzalez-Dunia¹, Stéphane Chavanas¹

¹Centre de Physiopathologie Toulouse Purpan, INSERM UMR 1043, TOULOUSE, France

²IUCT-Oncopole Toulouse, France, TOULOUSE, France

³Hôpital Necker-Enfants Malades AHP and Paris Descartes University, PARIS, France

⁴I-STEM / INSERM U861 / AFM and UEVE U861, EVRY, France

Congenital infection by human cytomegalovirus (HCMV) is a leading cause of permanent sequelae of the central nervous system, including sensorineural deafness, cerebral palsies or devastating neurodevelopmental abnormalities (0.1 % of all births). We recently showed that HCMV infection triggers the activity of PPAR gamma (PPARg), a transcription factor critical for the developing brain and required for efficient replication of HCMV, in neural stem cells (NSCs) and fetal brain.

To gain more insight on the impact of HCMV on host gene expression, we performed further immunohistological analyses of control or infected fetal brains and transcriptional profiling of infected NSCs. Immunohistological examination of infected brains detected HCMV immediate-early antigen (IE) expression and nuclear PPARg in all samples from infected cases. The number of infected and PPARg expressing neural progenitors was variable, independently from the gestational age. Brain samples from uninfected cases showed no detectable expression of HCMV antigens or PPARg. Transcriptional profiling of infected NSCs showed upregulation of the expression of genes related to antiviral cell response. Also, we found altered expression of a set of genes known to be involved in neurogenesis and / or cell mobility. In particular, genes critical for neural cell migration and brain development showed strongly increased expression in infected NSCs, at the mRNA and protein levels. Their upregulation was

found to be associated with PPAR γ in infected NSCs, or in uninfected NSCs with singled-out expression of PPAR γ . These results were supported by immunohistological analyses of infected fetal brain slices relative to control cases. Consistently, functional assays revealed impaired migration ability of infected NSCs in vitro. These findings indicate that impaired neural cell migration could underlie the neurodevelopmental abnormalities in congenital HCMV infection.

420 - Session B.05: Virus and Host

Date: 03/05/2017

Time: 10:55 - 13:05 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-018

A New Strain of Guinea Pig CMV Suggests a More Prudent Strategy for Evaluation of Interventions Against Congenital CMV

K. Yeon Choi, Alistair McGregor

Texas A&M University Health Science Center, COLLEGE STATION, United States of America

The guinea pig is the only small animal model for congenital CMV (cCMV) studies but requires the use of guinea pig CMV (GPCMV). We recently demonstrated conserved function and immunogenicity of the homolog viral glycoprotein complexes, including the pentameric complex (PC) necessary for epithelial tropism and congenital infection. A recent live GPCMV vaccine (pp65 knockout/PC+) was highly immunogenic and completely protected against cCMV. This was the first time that complete protection had been attained and was an important milestone. However, all GPCMV studies to date have utilized the original virus isolated in the 1950s and passaged multiple times on fibroblasts before being deposited with the ATCC. This virus (strain 22122) when serially passaged in animals and used as a salivary gland stock causes congenital infection. However, focusing only on this one strain of GPCMV may not provide a sufficiently comprehensive evaluation of pre-clinical vaccine strategies. This concern is raised by a new strain of GPCMV (CRV) which we isolated from animals. The growth kinetics, tropism and immunogenicity of the ATCC and CRV strains are very different. In contrast to 22122, CRV evoked a relatively low antibody titer immune response to the various GPCMV glycoprotein complexes (gB, gH/gL, gM/gN and PC), despite comparable normal level of anti-GPCMV antibody titers and similar protein sequences. Additionally, CRV was highly epithelial trophic (renal and trophoblasts), cell associated, and extremely difficult to propagate on fibroblasts. Adaptation for fibroblast growth results in loss of epi-tropism. In contrast, the full length ATCC GPCMV can be propagated on both epithelial and fibroblast cells. Various studies were carried out with ATCC and CRV strains in primary and re-infection models. Overall, cross reacting immune response reduced the incidence of cCMV. However, systemic dissemination of CRV virus in ATCC infected (sero-positive) animals or vice versa was not greatly impacted.

420 - Session B.05: Virus and Host

Date: 03/05/2017

Time: 10:55 - 13:05 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-019

Breast milk HCMV viral load drives the establishment of breast milk CMV-pp65-specific CD8 T cells in HCMV infected mothers.

Steffanie Sabbaj, David Moylan, Sunil Pati, Shannon Ross, Suresh Boppana

University of Alabama at Birmingham, Medicine, BIRMINGHAM, United States of America

Background: Immunologic correlates of HCMV transmission at the mucosal surface are not known. In spite of passively acquired maternal antibodies, about 50% of breastfed babies of seropositive mothers will acquire HCMV from breast milk (BM). Therefore, studying BM transmission of HCMV provides a

unique opportunity to investigate protective antiviral immune responses.

Objective: To examine the frequency of HCMV-specific T-cells and tissue resident memory T-cells (TrM) in peripheral blood mononuclear cells (PBMC) and breast milk cells (BMC) in HCMV-seropositive lactating women (N=45).

Methods: The frequency of CD4 and CD8 HCMV-pp65-specific T-cell responses in BM and PBMC was determined by measuring IFN- γ and dual IFN- γ /GrB production by intracellular cytokine staining using flow cytometry.

Results: The median frequency of HCMV-pp65-specific T cells is increased in both compartments (PBMC and BMC) for CD8+ T-cells (0.4% & 1.1%) when compared to CD4+ T-cells (0.02% & 0.25%). The frequency of HCMV-pp65-specific CD8 T-cells by the production of IFN- γ alone or dual IFN- γ /GrB was increased in BMC (1.3% & 1.3%) compared to PBMC (0.6% & 0.5%). A positive relationship between BM viral load and CD8 CMV-pp65-specific BMC or BM cell counts was observed. Lastly, we demonstrate the presence of TrM in BM. An inverse relationship between CD8 TrM cell frequency and BM viral load was observed, although not statistically significant ($p=0.08$).

Conclusions: Both CD4 and CD8 HCMV-specific T-cells were present in BM. A positive relationship between CD8 CMV-pp65-specific T-cells and viral load suggests that local virus replication drives antigen-specific CD8 T-cells into the breast. Also, the presence of TrM, a relatively new memory T-cell maintained in non-lymphoid tissue and capable of providing immediate protective immunity in tissues, maybe associated with the control of virus replication in BM. Together this data suggests that T-cells may play a role in HCMV control in BM.

420 - Session B.05: Virus and Host

Date: 03/05/2017

Time: 10:55 - 13:05 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-020

The UL40 homologue Rh67 is required for the induction of MHC-E-restricted CD8+ T cells by RhCMV

Klaus Frueh, Marieke Verweij, Isabel Scholz, Daniel Malouli, Colette Hughes, Alfred Legasse, Michael Axthelm, Scott Hansen, Louis Picker
Oregon Health & Science University, BEAVERTON, United States of America

Inoculation of rhesus macaques (RM) with rhesus CMV (RhCMV) strain 68-1 elicits unconventional CD8⁺ T cells restricted by MHC-II and MHC-E. The highly conserved, non-polymorphic MHC-E molecule is generally not involved in antigen presentation to T cells but acts as an inhibitory "self"-ligand for NK cells by presenting the peptide VMAPRTLII (VL9) derived from the signal sequence of classical, polymorphic MHC-I molecules. HCMV UL40 and RhCMV Rh67 encode a highly conserved VL9 peptide but otherwise demonstrate very little homology to each other or to MHC-I. To examine their role of Rh67 and UL40 in the induction of MHC-E restricted CD8+ T cells, we generated a series of RhCMV recombinants and examined their impact on MHC-E expression and T cell priming. We show that the VL9 sequence in Rh67 is required for maturation and cell surface expression of MHC-E in RhCMV-infected cells since in the absence of Rh67, or upon disruption of the VL9 sequence, MHC-E is retained intracellularly. Moreover, unlike native VL9 peptides, Rh67-derived VL9 does not require the peptide transporter TAP to support MHC-E maturation. We further demonstrate that priming of MHC-E restricted CD8+ T cells requires the Rh67-encoded VL9 peptide since single point mutations within VL9 completely abolish MHC-E restricted CD8+ T cell responses resulting in RhCMV that exclusively elicits T cells restricted by MHC-II. Importantly, HCMV UL40 can substitute for Rh67 both with respect to supporting MHC-E maturation *in vitro* and MHC-E-restricted T cells *in vivo*. Thus, priming of MHC-E-specific CD8+ T cells involves direct antigen presentation by RhCMV-infected cells and possibly the exchange of MHC-E-bound VL9 peptide for antigen-derived peptides. These results further suggest that Rh67 is required for the unique ability of RhCMV 68-1 to elicit unconventional T cells and that 68-1-like HCMV might elicit HLA-E restricted CD8+ T cells in humans.

440 - Session A.02: Epidemiology & Burden of Disease

Date: 03/05/2017

Time: 14:15 - 15:45 hrs

Invited presentation

A-INV-002

Congenital CMV infection: can we predict long-term outcome?

Ann Vossen

Leiden University Medical Center, LEIDEN, Netherlands

Congenital cytomegalovirus infection (cCMV) is the most common congenital infection worldwide with a birth prevalence of around 0,6 % in developed countries. The prevalence of long-term moderate-to-severe impairment in the group of children with cCMV has been estimated to be 17 % in a systematic review based on prospective studies and 25 %, with an attributable risk of 13 %, in the large retrospective CROCUS study. It is important to realise that half to two thirds of these children had no symptoms at birth. Universal neonatal screening could identify most neonates with cCMV, but has not been introduced. One of the reasons is the fact that only part of the children identified through universal screening will develop long-term sequelae. A reliable prognostic marker would support the introduction of neonatal screening. Most studies on prognostic markers have focused on children with cCMV with symptoms at birth, with a prenatal diagnosis (mostly after primary maternal infection) and on neuro-imaging. Using the large nation-wide retrospective cCMV cohort from the CROCUS study, we have studied several markers in the neonatal dried blood spots (DBS) in relation to long-term outcome. During my presentation I will show some of the challenges of working with DBS in this retrospective cohort and present some preliminary results.

440 - Session A.02: Epidemiology & Burden of Disease

Date: 03/05/2017

Time: 14:15 - 15:45 hrs

BOTH ORAL & POSTER PRESENTATION

A-OP-005

Neuroimaging findings as potential predictors of adverse outcomes in asymptomatic congenital CMV infection (cCMV) identified at birth

Aparecida Yulie Yamamoto, Adriana Carnevale da Silva, Sara Teixeira, Jorge Elias Junior, Myriam Lima Isaac, Adriana Ribeiro Anastasio, Eduardo Tanaka Massuda, Alessandra Kerli Manfredi, Marisa Marcia Mussi-Pinhata

Ribeirão Preto Medical School, University of São Paulo, Department of Pediatrics, RIBEIRÃO PRETO, Brazil

Background: The proportion of neuroimaging abnormalities at birth in asymptomatic infants with congenital CMV infection is not well defined because only the neonatal screening could identify infants without clinical abnormalities.

Objective: To determine the frequency and characteristics of neuroimaging abnormalities in asymptomatic infants with cCMV infection and to verify its association with the occurrence of hearing loss.

Methods: By means of a CMV neonatal screening, 66 of 11,950 infants were identified as congenitally infected. All infants underwent physical examination at birth, neuroimaging [brain transfontanellar ultrasonography (61/66; 92.4%) and/or cranial magnetic resonance (25/66; 37.8%)], ocular funduscopy, and hearing evaluation using evoked otoacoustic emissions and auditory brainstem response.

Results: Among 66 newborns, 8 (12%) presented one or more clinical findings of congenital infection and 58 (89%) were apparently asymptomatic at birth. Of these, 52 (91.4%) underwent complete evaluation within 3 months of life. Unilateral chorioretinitis was observed in one infant (2 %).

Neuroimaging abnormal findings were observed in 29/52 (55.7%) infants. The most common findings were lenticulostriate vasculopathy with subependymal pseudocysts (13/29 (43.3%); isolated subependymal pseudocysts (11/29 (37.9%); single or periventricular calcifications and/or gliosis (4/29 (13.8%); and ventriculomegaly (1/29 (3.4%). Unilateral profound hearing loss at birth was found in 4/52

(7.7%) infants and neuroimaging findings were observed in 3 of these infants (75.0%) while 26 (54.2%) of 48 infants with no hearing loss had abnormal imaging features (p=0.28).

Conclusions: Although a neonatal screening of cCMV will identify the majority of infected infants who are clinically asymptomatic, a significant proportion of them could benefit from a central nervous system image evaluation, since abnormal findings are frequent. Neuroimaging could be a potential prognostic marker of adverse outcomes of congenital CMV such as hearing loss in asymptomatic infants.

440 - Session A.02: Epidemiology & Burden of Disease

Date: 03/05/2017

Time: 14:15 - 15:45 hrs

BOTH ORAL & POSTER PRESENTATION

A-OP-006

Outcomes From a State-wide Hearing-Targeted Cytomegalovirus Screening Program

Albert Park¹, Marissa Diener¹, Zick Cathy¹, Stephanie McVicar², Jill Boettger³

¹University of Utah, SALT LAKE CITY, United States of America

²Dept of Health, SALT LAKE CITY, United States of America

³Department of Health, SALT LAKE CITY, United States of America

BACKGROUND AND OBJECTIVES: Cytomegalovirus (CMV) is the most common congenital infection abstract and nongenetic cause of congenital sensorineural hearing loss in the United States. Utah was the first state to pass legislation mandating CMV screening for newborns who fail newborn hearing screening (NBHS). The study objective was to present outcomes of hearing-targeted CMV screening and determine factors predicting CMV screening. **METHODS:** We used Utah Department of Health HiTrack and Vital Records databases to examine CMV screening from 509 infants who failed NBHS in the 24 months after implementation of the Utah legislation. Multivariate logistic regression analyses were conducted to identify predictors of compliance with CMV screening and diagnostic hearing evaluation. **RESULTS:** Sixty-two percent of infants who never passed hearing screening underwent CMV screening. Fourteen of 234 infants tested within 21 days were CMV positive; 6 (42.9%) had hearing loss. Seventy-seven percent of eligible infants completed a diagnostic hearing evaluation within 90 days of birth. Compliance with CMV screening was associated with sociodemographic factors, time since the law was enacted, and NBHS protocol. Infants born after the legislation showed greater odds of achieving timely diagnostic hearing evaluation than infants born before the law. **CONCLUSIONS:** Incorporating CMV screening into an established NBHS program is a viable option for the identification of CMV in infants failing NBHS. The addition of CMV testing can help a NBHS program attain timely audiological diagnostics within 90 days, an important early hearing detection and intervention milestone.

440 - Session A.02: Epidemiology & Burden of Disease

Date: 03/05/2017

Time: 14:15 - 15:45 hrs

BOTH ORAL & POSTER PRESENTATION

A-OP-007

Infectious Reactivation of Cytomegalovirus Explaining Age- and Sex-Specific Patterns of Seroprevalence

Michiel Van Boven¹, Jan Van de Kasstelee¹, Marjolein Korndewal¹, Christiaan Van Dorp², Fiona Van der Klis¹, Hester De Melker¹, Mirjam Kretzschmar¹, Ann Vossen³, Debbie Van Baarle¹

¹National Institute for Public Health and the Environment, BILTHOVEN, Netherlands

²Utrecht University, UTRECHT, Netherlands

³Leiden University Meidcal Center, LEIDEN, Netherlands

Human cytomegalovirus (CMV) is a herpes virus with poorly understood transmission dynamics. Person-to-person transmission is thought to occur primarily through transfer of saliva or urine, but no

quantitative estimates are available for the contribution of different infection routes. We here use data from a population-based serological study to provide quantitative estimates of the transmissibility of primary infection, reactivation, and re-infection. Mixture models are fitted to age- and sex-specific antibody response data, showing that the data are well described by a model with three distributions of antibody measurements, viz. low (uninfected), intermediate (infected), and high (infected with increased antibodies). Estimates of seroprevalence and fraction of the population with raised antibodies increase gradually with age, such that at 80 years 73% (95%CrI: 64%-78%) of females and 62% (95%CrI: 55%-68%) of males is infected, while 57% (95%CrI: 47%-67%) of females and 37% (95%CrI: 28%-46%) of males has experienced a reactivation or re-infection episode. Merging the statistical analyses with transmission models, we find that models with infectious reactivation (i.e. reactivation that can lead to the virus being transmitted to a novel host) fit the data significantly better than models without infectious reactivation. Estimated reactivation rates increase from low values in children to 2%-6% per year in women older than 50 years. The results advance a hypothesis in which adult-to-adult transmission during infectious reactivation is a key driver of infection. We discuss the implications for control strategies, including vaccination, aimed at reducing CMV infection in vulnerable groups.

440 - Session A.02: Epidemiology & Burden of Disease

Date: 03/05/2017

Time: 14:15 - 15:45 hrs

BOTH ORAL & POSTER PRESENTATION

A-OP-008

Increasing Awareness and Promoting Strategies for Prevention of Congenital Cytomegalovirus Infection (cCMV) Among Young Pregnant Women

Karen Fowler¹, Susan Davies¹, Mirjam Kempf¹, Suresh Boppana¹, Michael Cannon², Alan Tita¹, Rodney Edwards³

¹University of Alabama at Birmingham, Pediatrics, BIRMINGHAM, United States of America

²National Center on Birth Defects & Developmental Disabilities, CDC, ATLANTA, United States of America

³Obstetrics & Gynecology, University of Oklahoma Health Science Center, OKLAHOMA CITY, United States of America

Background: Previous data indicate that most women have never heard of cCMV and the risk of cCMV's damaging sequelae for their newborns, or how to prevent CMV exposures.

Objective: To evaluate whether a cognitive-behavioral intervention can, 1) increase knowledge about cCMV and 2) decrease self-reported risk behaviors.

Methods: Young pregnant women were recruited into a CMV cognitive-behavioral intervention study following their first prenatal visit and 215 women (16 – 29 yrs) were randomized to either a CMV educational/prevention intervention (PREV) or an attention-matched control using an educational stress reduction intervention (CONT). Both groups attended an individualized behavioral skills session, watched a short video, received a take home packet, received weekly text messages for 12 weeks to deliver the experimental and control interventions, and attended 6 and 12 week follow up visits. Pre- and post-intervention CMV knowledge and CMV risk behaviors were assessed via questionnaires in both groups.

Results: Pre- and post-intervention assessments were completed in 196 women (91.2%). The cohort was 91% Black, with 75% being CMV seropositive. Only 14.2% (95% CI, 9.7 – 19.8%) of the women had ever heard of cCMV at study enrollment and their mean (\pm SD) CMV risk behavior score was 5.5 ± 6.1 (possible range 0 – 32). Post intervention, the mean correct CMV knowledge (possible scale 0 – 16) was 11.3 ± 2.2 in the PREV group compared to the CONT group (8.5 ± 3.7 , <0.0001). Also, post-intervention, the PREV women reported a lower mean CMV risk behavior score (1.7 ± 2.6) compared to the CONT women (3.4 ± 4.6 , $p=0.002$).

Conclusion: Young women lack awareness or accurate knowledge of cCMV and how to protect themselves and their fetuses/infants from CMV infection. This intervention demonstrates that it is possible to raise awareness about cCMV and decrease CMV risk behaviors in young pregnant women.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-028

Valnoctamide reverses CMV-mediated brain injuries and neurological deficits in newborn mice

Sara Ornaghi¹, Patrizia Vergani², Anthony Van den Pol³, Michael Paidas³

¹University of Milan-Bicocca, Yale University, Obstetrics and Gynecology, Neurosurgery, MONZA, Italy

²University of Milan-Bicocca, MONZA, Italy

³Yale University, NEW HAVEN, United States of America

OBJECTIVE: Congenital CMV represents the leading viral cause of brain defects and can lead to life-long debilitating neurological problems. No drugs are currently approved for in-utero treatment of infected fetuses due to potential teratogenicity, and brain injuries occurred during fetal development are unlikely reversed by postnatal therapies. We showed that valnoctamide (VCD), a clinically available mood stabilizer, effectively blocks CMV. Here we investigate the anti-CMV activity of VCD in the brain of infected newborn mice and the potential benefits of VCD treatment on neurological outcomes. **DESIGN:** Pups inoculated intraperitoneally with murine CMV (mCMV, 750 PFU) on the day of birth received VCD or vehicle daily (1.4mg/mL) from PND1 to PND21. Immunofluorescence and qPCR were employed to assess brain pathology and viral load, respectively. Brain/body weight ratio was measured at P30 to analyze brain growth. Neurobehavioral ontogeny during neonatal period was investigated by righting and grasping reflex, cliff aversion, and negative geotaxis. Pole test and challenging beam traversal test allowed evaluation of motor development in adolescent mice. **RESULTS:** CMV was detected as early as 4 day post-inoculation in multiple regions of the brain, with viral load peak at P12. Analysis of P14 and P30 infected brains revealed disrupted hippocampus and cerebellum, with progressive neuronal loss, and deficient brain growth compared to uninfected controls. Delayed appearance of neurological milestones and motor impairment were identified in infected neonates and adolescent animals. VCD administration substantially decreased mCMV load in the CNS ($p < 0.0001$), and rescued both viral-induced pathology and deficient growth of the brain ($p < 0.001$), with significant improvement in neurobehavioral ontogeny and motor development of infected mice. **CONCLUSION:** VCD blocks CMV brain infection and rescues the viral-mediated abnormal neurological outcomes. Since VCD is available on the market and lacks teratogenicity, it may merit consideration in treating CMV during early development.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-006

Cytomegalovirus-related polymicrogyria in seroimmune pregnant women

Maurizio Zavattoni¹, Giuseppina Lombardi¹, Micol Angelini¹, Guido Scalia², Andrea Righini³, Milena Furione¹, Fausto Baldanti⁴

¹IRCCS Policlinico San Matteo, Microbiology and Virology, PAVIA, Italy

²A.O.U Policlinico-Vittorio Emanuele, O.P., Gaspare Rodolico, Clinical Virology Unit, CATANIA, Italy

³Buzzi Children's Hospital, Pediatric Radiology and Neuroradiology, MILAN, Italy

⁴University of Pavia, Clinical, Surgical, Diagnostic and Pedia, PAVIA, Italy

Background. Primary maternal HCMV infection during pregnancy is a critical determinant of intrauterine transmission and outcome. On the other hand, in developed countries maternal-to-fetal transmission risk in non-primary infection seems to be much lower. Moreover, severe symptoms at birth and long-term sequelae are seen more frequently among children from preconceptionally seronegative than seropositive women, supporting the hypothesis that acquired maternal immunity provides substantial protection from congenital disease.

Patients and methods. We describe five cases of symptomatic congenital HCMV infection with brain

abnormalities in seroimmune mothers. At that time, primary HCMV infection in pregnancy was excluded by specific IgM negative with HCMV IgG positive within the first trimester of gestation (range 6-9 weeks). In one case, increasing HCMV IgG titre with specific IgM appearance in pregnancy were observed. No IgG avidity data were available. All pregnant women were considered immune for HCMV and therefore no educational and hygienic information was offered. Congenital HCMV infection was documented by virus detection in urine and blood at birth in three cases and retrospectively by viral DNA detection in dried-blood-spot specimen in one case.

Results. In four cases congenital HCMV infection was suspected due to clinical presentation at birth (IUGR, microcephaly, abnormal movements, hyperexcitability, increased periventricular echogenicity). In one case, despite microcephaly at birth, maternal immunity for HCMV was misleading and HCMV congenital infection was postulated only three years later. All newborns presented with white matter abnormalities and polymicrogyria/pachygyria at brain magnetic resonance imaging

Conclusions. Although primary HCMV infection cannot be formally excluded, maternal non-primary infection appears most probable. There is a need to better understand: (1) the frequency with which non-primary infections result in disabilities; (2) the attributable fraction of non-primary infections caused by reactivation or re-infection with a different HCMV strain; and (3) the effectiveness of hygienic measures to prevent non-primary HCMV infection in pregnancy

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-007

A rare case of periconceptual congenital cytomegalovirus infection in a dichorionic twin pregnancy

Verena Kuret, Christine Osborne, Emma Curran, Eliana Castillo, Stephanie Cooper
University of Calgary, Department of Obstetrics & Gynecology, CALGARY, Canada

This case presents a rare finding of discordant CMV infection in a dichorionic twin pregnancy caused by periconceptual maternal CMV infection. A healthy 35-year-old G5P3A1L3 presents for a routine anatomical scan for a dichorionic diamniotic twin pregnancy which identifies in fetus 1: severe intrauterine growth restriction (IUGR), oligohydramnios, microcephaly, mild ventriculomegaly, small fetal kidneys, echogenic bowel, and ascites. A subsequent fetal MRI identifies additional brain anomalies including periventricular echogenic foci, cerebellar hypoplasia, possible polymicrogyria and schizencephaly. Ultrasound and MRI monitoring of the co-twin (fetus 2) shows no anomalies. Maternal CMV serology on multiple blood samples showed no seroconversion in pregnancy and CMV avidity testing was suggestive of a remote infection occurring 4-8 weeks prior to conception. Further invasive testing to investigate for congenital CMV infection was therefore not completed. Two live infants were delivered via planned cesarean delivery at 37+5 weeks. Postnatal urine and cord blood testing of the twins revealed an unexpected CMV infection in fetus 1. Imaging, cord blood immunoglobulin and urine CMV PCR for fetus 2 was negative, with no evidence of other abnormalities.

Transplacental viral transmission of CMV can occur following a primary or recurrent infection. Fetal transmission rates and effects on the fetus vary with gestational age at infection. This case illustrates pre-conception or periconceptual maternal CMV infection can be associated with severe congenital anomalies. However, little information exists about the specific transmission rates with this type of infection. Furthermore, current CMV avidity testing gives an imprecise range of presumed infection and can be falsely reassuring as seen in this pregnancy. This case was further complicated by the discordant findings, with only one twin infected with CMV. When ultrasound and MRI findings are highly suggestive of fetal infection, in the setting of negative CMV serology, periconceptual CMV infection must be considered and confirmed with amniocentesis.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
CD-PP-014

Prospective monitoring for CMV DNAemia in VLBW infants identifies unrecognized but clinically significant breast milk-acquired infections in high-risk NICU patients

Mark Schleiss¹, MaryPat Osborne², Nelmary Hernandez-Alvarado¹, Erin Osterholm²

¹Centre for Infectious Diseases and Microbiology Translational Research, University of Minnesota Medical School, Department of Pediatrics, MINNEAPOLIS, United States of America

²Division of Neonatology, University of Minnesota Medical School, Department of Pediatrics, MINNEAPOLIS, United States of America

The short and long-term risks associated with post-natal CMV acquisition in premature infants remain largely unknown. Preterm very low birth weight (VLBW) infants in the newborn intensive care unit (NICU) are at high risk for the development of symptomatic CMV disease from breast milk. We examined the prevalence of virolactia, CMV transmission rates, and occurrence of symptomatic CMV disease in a prospective analysis of 51 mother-infant dyads at the University of MN Masonic Children's Hospital NICU. Infants were <1500 grams and without congenital CMV infection. Weekly samples of breastmilk and blood were analyzed for CMV via qPCR. Serum samples were also tested for CMV antibodies by ELISA. Viral load analyses of breast milk and blood identified shedding of CMV virus in milk in 14/39 mothers (rate of 36%). Most lactating mothers with CMV IgG antibodies (83%) demonstrated virolactia during the surveillance period. Median viral load was 7.8×10^3 copies/ml milk. Two infants from the 14 exposed to CMV in breast milk (14%) developed CMV DNAemia, with a mean viral load of 276 copies/mL of blood. The healthcare team did not identify CMV infection during the hospital course. Both courses were complicated by the need for mechanical ventilation, with one infant meeting the criteria for chronic lung disease, requiring supplemental oxygen at discharge. This infant was also treated for gram negative pneumonia. In summary, over 1/3rd of mothers in our sample (and >80% of these with CMV IgG antibodies) had active shedding of CMV in breast milk. Post-natally infected infants in this prospective analysis had chronic lung disease and new-onset bacterial pneumonia, consistent with studies demonstrating increased rates of chronic lung disease and gram negative sepsis/infections in VLBW infants with acquired CMV. Prospective surveillance for CMV DNAemia should be considered as a component of routine care in breast-fed NICU infants.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
CD-PP-020

Association between Cytomegalovirus (CMV) viral load in fetal blood and antenatal imaging anomalies

Elena Costa¹, Marie-Luce Delforge¹, Siham Zaytouni¹, Françoise Brancart¹, Deborah Goldman¹, Isabel Montesinos¹, Arnaud Marchant², Catherine Donner¹

¹Erasme University Hospital, Obstetrics and Gynaecology, ANDERLECHT, Belgium

²Institute for medical immunology, GOSELIES, Belgium

Background: A newborn vertically infected with Cytomegalovirus (CMV) has a 15 to 20 % risk of developing sequelae. Antenatal imaging anomalies on ultrasound (US) or Magnetic resonance (MRI) are associated with poor prognosis. Quantification of CMV viral load (VL) in fetal blood is a potential marker of the development of imaging anomalies.

Objectives: To investigate the relationship between the CMV VL in fetal blood and the presence of antenatal imaging anomalies.

Study design: Quantification of CMV DNA was performed on 46 positive fetal blood samples from women with proven CMV primary infection, reactivation or reinfection. We used Wilcoxon Mann-Whitney test to assess the relationship between CMV VL (expressed in copies/ml) and presence of imaging anomalies. We subsequently separately assessed severe US brain anomalies, mild US brain anomalies, extracerebral US anomalies, severe MRI anomalies and mild MRI anomalies.

Results: Median CMV VL was higher in those fetuses presenting with any imaging anomaly (Med 45650 IQR 11550-548000) versus those presenting no anomaly (Med 9680, IQR 4630-42200), $p = 0.07$; we observed similar results in severe US brain anomalies vs no anomalies (Med 228500, IQR 61300-1061640 vs Med 9680, IQR 4630-42200, $p=0.08$), mild US brain anomalies vs no anomalies (Med 32100, IQR 12100-46200 vs Med 9680, IQR 4630-42200, $p=0.18$), extracerebral US anomalies vs no anomaly (Med 72900, IQR 12100-862000 vs Med 9680, IQR 4630-42200, $p= 0.056$) severe MRI anomalies vs no anomaly (Med 53100, IQR 22700-304950 vs 17800, IQR 7110-42200, $p= 0.27$) and mild MRI anomalies vs no anomalies (Med 39750, IQR 11000-559000 vs 17800, IQR 7110-42200, $p= 0.37$) .

Conclusion: We found a higher VL in fetuses presenting with any kind of investigated imaging anomalies. None of the relationships highlighted reached statistical significance, but the consistency among them signals that it might be because of our small number of subjects.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-021

Congenital Cytomegalovirus saliva screening triggered by repeated failure on newborn hearing screening - a pilot study

Horst Buxmann¹, Lena Schütz¹, Rolf Lambert Schlößer¹, Klaus Hamprecht², Holger Rabenau¹, Lara Pfisterer¹, Sabine Kramer¹

¹University Hospital Frankfurt, Pediatrics - Neonatology, FRANKFURT/MAIN, Germany

²University Hospital, Medical Virology, TÜBINGEN, Germany

Introduction

Nonhereditary sensorineural hearing loss is predominantly caused by congenital Cytomegalovirus (cCMV) infection. Currently no universal cCMV-screening exists in Europe. This study was initiated to analyse whether a cCMV saliva screening induced by repeated referral on newborn hearing screening (NHS) may help to identify the most suitable candidates for antiviral therapy.

Methods

This ongoing prospective interventional trial includes newborns within 21 days of life, who failed their NHS twice to identify potential cCMV-infection. A saliva specimen is tested by means of liquid-saliva CMV-PCR-assay. Positive CMV-PCR results lead to confirmatory CMV-tests on urine, blood and dried-blood-spot (DBS), as well as physical examinations and head-ultrasounds.

Results

From 03.08.2015–31.12.2016 on 1114 patients a control-NHS was performed at the pediatric audiology of the University Hospital Frankfurt. Overall 882 (79.6%) passed and 232 (20.4%) failed this control-NHS. Of the latter 172 (74.1%) were older than, and 60 (25.9%) within 21 days of life. 58 newborns were included in our study yet. Two (3.4%) showed CMV-PCR positive saliva specimen. Both were additionally CMV-PCR positive in urine, blood and DBS. The CMV-PCR of 20 additional DBS available so far, were negative, consistent with the saliva specimen of these children.

Discussion

The presented algorithm is capable to identify newborns with hearing impairment and cCMV-infection. We found an incidence lower than in published data (20%). The majority of our cohort has a normal control-NHS or is older than 21 days at their control-NHS. The latter cannot be included in our study because it is impossible to differentiate between congenital and postnatal CMV-infection beyond that age.

Conclusion

If a control-NHS takes place within 21 days of life, our study algorithm is a valuable tool to identify those cCMV-infected newborns that benefit of an antiviral therapy.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
CD-PP-022

Significant association between Human Cytomegalovirus-IEA and expression of cyclooxygenase-2 and 5-lipoxygenase in human breast cancer specimens

Afsar Rahbar¹, Helena Costa¹, Joel Touma¹, Belghis Davoudi¹, Ida Rashid Bukholm², Torill Sauer³, Jürgen Geisler³, Cecilia Söderberg Naucner¹, Katja Vetvik⁴, Afsar Rahbar¹

¹Karolinska Institutet, STOCKHOLM, Sweden

²Norwegian system of compensation for patient claimers and Norwegian University of, OSLO, Norway

³Department of Oncology at AHUS, Department of surgery and oncology (AHUS), and I, OSLO, Norway

⁴Department of Breast and Endocrine Surgery at Akershus University Hospital (AHU, OSLO, Norway)

Background: Cyclooxygenase-2 (COX-2) and its derived metabolites, 5-lipoxygenase (5-LO) and leukotrienes have been associated with breast cancer (BC) progression and metastasis. Human Cytomegalovirus (HCMV) detection in samples from primary BC, sentinel lymph nodes and brain metastases obtained from breast cancer patients' suggests that viral infection may also have a critical role in the development of BC metastasis. Interestingly, *in vitro* studies showed that HCMV infections induce COX-2 which augments viral replication through a prostaglandin dependent pathway. Thus, our main objective was to investigate whether there is a correlation between HCMV infection and expression of COX-2 and 5-LO in BC. If so, HCMV could be an important additional target for breast cancer treatment.

Material and Methods: Paraffin embedded breast cancer biopsies (n=49), ductal carcinoma in-situ (DCIS, n=14) and adjacent, benign breast tissue samples (n=26) were retrospectively examined for HCMV-immediate early (IE), HCMV-Late (LA) proteins, COX2 and 5LO by using immunohistochemical techniques. All patients received standard adjuvant treatment.

Results: High levels of COX-2, 5-LO and HCMV-IE were detected mainly in breast cancer samples. High grade HCMV-IE (defined as >50% positive cells in the tumor tissues) was detected in 72% of infiltrating BC and in 36% of DCIS, but it was detected only in 8% of benign, adjacent breast tissue samples. Similarly, high grade COX-2 and 5-LO were detected in 58% and 73% of BC, in 29% and 17% of DCIS, and in 8% and 31% of benign, adjacent breast tissue samples, respectively. We found a statistically significant positive correlation for the levels of HCMV-IE and COX-2 ($p=0.001$) as well as for HCMV-IE and 5-LO ($p=0.0002$) in infiltrating breast cancer.

Conclusion: Our findings confirm a positive correlation of HCMV-IE protein synthesis and overexpression of COX-2 and 5-LO in infiltrating breast cancer, DCIS and benign, adjacent breast tissue samples.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
CD-PP-023

Ovarian Cancer Survival is Associated with detection of Human cytomegalovirus infection in the tumor

Afsar Rahbar¹, Angeliqe Floter Radestad², Atosa Esteki Zadeh¹, Belghis Davoudi¹, Angelica Lindén Hirschberg², Joseph Carlson³, Cecilia Söderberg-Naucner¹, Afsar Rahbar¹

¹Karolinska Institutet, STOCKHOLM, Sweden

²Department of Women's and Children's Health, Karolinska University Hospital, STOCKHOLM, Sweden

³Department of Pathology and Cytology and Institute for Oncology-Pathology, STOCKHOLM, Sweden

Background: Ovarian cancer is a significant cause of death in women worldwide with unknown aetiology. Human cytomegalovirus (HCMV) has recently been detected in breast cancer and other tumors with epithelial origin. Since breast- and ovarian cancer are linked through BRCA gene mutations and have morphological similarities, we aimed to study the prevalence of HCMV protein expression in ovarian cancer and relate the infection grade to clinical outcome.

Material and Methods: In a prospective study, we collected blood samples and paraffin embedded tissue sections obtained from patients with ovarian cancer (n=45), borderline (n=13) and benign ovarian tumours (n=30), and examined tumor tissue specimens for HCMV- immediate-early protein (IE), HCMV tegument protein (pp65) by immunohistochemistry staining, and serum for HCMV serology.

Results: High grade HCMV-IE and pp65 (defined as >50% positive cells within the tissue section) were detected in 73%, 85%, 67% and in 26%, 38% and 14% of patients with ovarian cancer, borderline and benign tumors, respectively. Among ovarian cancer patients with high grade HCMV-pp65 protein expression in their tumors 27% were alive at study closure, as compared with 58% of patients with low grade HCMV-pp65 expression ($p=0.03$). The levels of HCMV-IgG were significantly higher in patients with ovarian cancer, borderline and benign ovarian tumours; compared with age matched healthy women ($p=0.001$, $P=0.008$, $p<0.0001$, respectively) and only ovarian cancer patients were positive for HCMV-IgM, (12%).

Conclusion: HCMV proteins are frequently detected at different levels in ovarian cancer, borderline and benign ovarian tumors. Ovarian cancer patients had detectable HCMV-IgM and higher HCMV- IgG levels was detected in patients with ovarian cancer, borderline tumors and benign tumors compared to healthy women. The survival was shorter among ovarian cancer patients with high grade HCMV-pp65 in their tumours, which high-light a need for further in depth studies of the role of HCMV in ovarian cancer.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-025

High prevalence of cytomegalovirus infection in surgical intestinal specimens from infants with necrotizing enterocolitis and spontaneous intestinal perforation

Afsar Rahbar¹, Soley Omarsdottira¹, Margret Agnarsdottir², Charlotte Casper³, Abiel Orrego⁴, Mireille Vanpée⁵, Afsar Rahbar¹, Cecilia Söderberg-Nauclér¹

¹Karolinska Institutet, STOCKHOLM, Sweden

²BDepartment of Immunology, Genetics and Pathology, Uppsala University, UPPSALA, Sweden

³Unit of Neonatology, Children's Hospital, Paul Sabatier University, TOULOUSE, France

⁴Departments of Clinical Pathology and Cytology, Karolinska University Hospital, STOCKHOLM, Sweden

⁵Women's and Children's Health, Karolinska University Hospital, STOCKHOLM, Sweden

Necrotizing enterocolitis (NEC) is a severe, often fatal gastrointestinal emergency that predominantly affects preterm infants, and there is evidence that neonatal cytomegalovirus (CMV) infection may in some cases contribute to its pathogenesis. Seventy intestinal specimens from 61 infants with NEC, spontaneous intestinal perforation (SIP), or related surgical complications were collected at Karolinska University Hospital and Uppsala University Hospital, Sweden. Ten specimens from autopsied infants without bowel disease served as controls. Samples were analyzed for CMV immediate-early antigen (IEA) and CMV late antigen (LA) by immunohistochemistry (IHC). In 10 index samples, CMV DNA was analyzed with Taqman PCR after laser capture microdissection (LCM) of cells positive for CMV IEA by IHC. Thirteen IHC-IEA-positive and 5 IHC-IEA-negative samples were analyzed for CMV DNA by in situ hybridization. The median gestational age was 27.9 weeks in index infants and 25.0 weeks in controls. CMV IEA was detected by IHC in 57 (81%) and CMV LA in 45 (64%) of 70 intestinal specimens from index cases; 2 (20%) of 10 control specimens were positive for both antigens. CMV DNA was detected in 4 of 10 samples (40%) after LCM. By in situ hybridization, all 13 IHC-IEA-positive samples were positive for CMV DNA; however, 3 of 5 IHC-IEA-negative samples (60%) were also positive. We conclude that CMV-specific antigens and CMV DNA were highly prevalent in intestinal specimens from infants with NEC, SIP, and related surgical complications. Our findings provide further evidence that neonatal CMV infection contributes to the pathogenesis of these diseases and may affect patient outcome.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-031

Comparison of culture from urine and PCR after 2 different extraction methods from DBS in clinically suspected for cCMV neonates

Elizaveta Padalko, Annelies Keymeulen, Maaïke Godefroid, Patrick Taveirne, Koenraad Smets
Ghent University Hospital, Laboratory Medicine, GHENT, Belgium

Background. Remaining the reference method for the diagnosis of congenital cytomegalovirus infection (cCMV) viral culture is more and more abandoned mainly due to pre-analytical issues. Detection of CMV DNA in dried blood spots (DBS) has been successfully used for the delayed diagnosis of cCMV in child with a hearing loss. As DBS are taken from every neonate born in Belgium, this specimen type can be theoretically ideal for the diagnosis of cCMV in neonates.

Objectives and methodology. To evaluate the value of PCR from DBS as compared to viral culture from urine in neonates suspected for cCMV, DBS and urine samples were collected following common protocol from 6 neonatal units in Flanders, Belgium. In order to investigate a potential difference in extraction efficacy, 2 extraction methods (Qiagen and easyMAG, bioMérieux) were performed prior to application of realtime in-house CMV PCR.

Results. A total of 286 results are available. Forty eight (16.8%) neonates were positive by viral culture on urine. For 45 (93.8%) of these patients, PCR on DBS after Qiagen extraction and for 47 (97.9%) of these patients PCR on DBS after easyMAG extraction were positive. For 4 additional patients with negative viral culture on urine, PCR on DBS after Qiagen extraction was positive; for 5 additional patients with negative viral culture on urine, PCR on DBS after easyMAG extraction was positive and only for 2 additional patients with negative viral culture on urine PCR's after both extractions were positive.

Conclusions. Despite the fact that 11 additional samples were found positive by PCR on DBS as compared to viral culture from urine, only 2 (18.2%) of these samples were consistently positive by PCR performed after 2 different extractions. These findings accentuate the importance of keeping in mind that the extraction method can strongly influence the final result.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-034

Comparison of H-DiaCMVQ (Diagenode) and CMV R-gene (Biomérieux) for CMV viral load quantification in whole blood specimens

Sébastien Hantz, Benjamin Crespy, Melissa Mayeras, Sophie Alain
Limoges University/Hospital; CNR des Cytomegalovirus, LIMOGES, France

Objectives: CMV viral load quantification has become a standard practice for follow-up of transplant patients. We evaluated the H-DiaCMVQ kit versus CMV R-gene. The performances were evaluated on whole blood (WB) samples and on CMV strains of varied genotypes.

Material and methods: 117 WB samples from transplant patients were collected (34 positive and 83 negative samples with R-gene). A CMV-DNA positive WB (between 5 and 6 log₁₀ copies/mL) was diluted in negative CMV WB to obtain a decreasing dilution range. DNA was extracted with Easymag (Biomérieux), then amplified on a Rotorgene thermocycler (Qiagen). Both PCR were processed from the same DNA extract within a maximum of 24 hours. As H-DiaCMVQ PCR targets the glycoprotein B gene, 12 strains of genotype 1, 2, 3a and 4a and recombinant 3/2 were tested (after DNA extraction according to the Hirt technique).

Results: Among the 117 clinical WB samples, qualitative correlation showed only 4 discordant samples. Kappa coefficient was 0.91 indicating an excellent agreement between both tests. Moreover,

discordances were observed for viral loads below the quantification threshold of the CMV R-gene. Quantitative correlation is good ($R^2 = 0.8$). Bland-Altman analysis confirmed that the results were not dispersed (97% of the samples between ± 2 SD) and showed an average difference of $1.6 \log_{10}$ between both techniques with an over-quantification for H-DiaCMVQ. The assay was linear over the range $2.7-4.8 \log_{10}$ copies/mL. All CMV genotypes were amplified by H-DiaCMVQ. Evaluation of conversion factor between copies/mL and IU/mL is ongoing.

Conclusion: The sensitivity and specificity of H-DiaCMVQ appeared to be quite satisfactory. A trend towards over-quantification has also been objectified. However, no false positives were induced by this technique. Finally, the target chosen by the manufacturer (gene of glycoprotein B) does not seem to affect the sensitivity compared to the PCRs targeting the *UL83* gene

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-035

Congenital CMV infection gives rise to a complex pattern of difficulties

Eva Karltorp, Ulrika Lofkvist, Mona-Lisa Engman, Ilona Lewensohn Fuchs
Karolinska Institutet, ENT dept, STOCKHOLM, Sweden

Although cytomegalovirus (CMV) is the most common congenital infection, existing research has not provided us with a full picture of how this can affect children in the future. The aim of this case-control study was to evaluate disabilities in a well-defined group of children with congenital cytomegalovirus (CMV) infection, who had been fitted with cochlear implants because of severe hearing impairment.

METHODS:

26 children with congenital CMV infection were assessed by a multidisciplinary team for balance difficulties, neurodevelopmental disabilities, language delay and visual impairment. 13 children with severe hearing impairment due to connexin 26 mutations served as control group.

RESULTS:

The majority of the children with congenital CMV infection (88%) displayed balance disturbances, including walking at a later age, but there were no cases in the control group. The CMV group also displayed frequent neurodevelopmental disabilities, visual impairment and feeding difficulties.

CONCLUSION:

Congenital CMV infection affects the general development of the brain and gives rise to a complex pattern of difficulties. Identifying comorbid conditions is very important, as children with associated difficulties and disabilities need more support than children with just hearing impairment. Congenital CMV infection needs to be considered in children with hearing impairment and/or balance disturbance and/or neurodevelopmental disabilities.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-036

Treatment of congenital CMV in Europe: From Chaos to Collaboration

Suzanne Luck¹, Daniel Blázquez Gamero², Francesca Garofoli³, Giuseppina Lombardi³, Vana Papaevangelou⁴, Schuster Katharina⁵, Philipp Henneke⁵

¹UCL centre for virology, LONDON, United Kingdom

²Hospital 12 de Octubre., Department of Pediatrics, MADRID, Spain

³Fondazione IRCCS Policlinico San Matteo, Neonatal Immunology, PAVIA, Italy

⁴General University Hospital ATTIKON, Third Department of Pediatrics, ATHENS, Greece

⁵Medical Center - University of Freiburg, Department of Pediatrics and Adolescent, FREIBURG,

Germany

Introduction:

Antiviral agents have been used to treat cytomegalovirus infection for decades. Optimal treatment regimens and efficacy in preventing long-term sequelae associated with congenital CMV (cCMV) infection remain, however, poorly defined. Side effects, including potential carcinogenicity, inhibit injudicious use of treatment.

Consequently many European countries have set up local or national databases in order to monitor treatment trends and outcomes in babies with cCMV.

Methods:

cCMV databases in place in 5 European countries were reviewed and data from babies born between 2007 and 2013 interrogated for trends in management and treatment.

Results:

Database aims were similar and definitions of symptomatic infection largely consistent although data actually collected varied widely. Some databases only recorded select groups e.g. 'symptomatic' or 'treated' babies. Data were available for 414 babies (43% 'symptomatic'); 14% were diagnosed antenatally. Baseline investigations, including cranial ultrasound, were conducted in most cases. MRI was conducted in 4%-48% of cases with no notable increase in later years. 223 babies received treatment with valganciclovir (single therapy or combined courses with ganciclovir) emerging as the favoured treatment in recent years. Treatment duration varied widely between countries with longer treatment courses being favoured in both Spanish and Italian cohorts. Viral load recording and long term follow-up data were limited.

Conclusions:

Although there were many similarities there were some notable differences between cohorts, primarily in onset of treatment and treatment duration. There was a predominance of symptomatic babies and combining data was limited by data recording differences. These issues are being addressed by the development of a shared database for all cCMV cases for use in centers throughout Europe and proposed unification and sharing of data outputs where feasible in established cohorts. Key hearing and neurodevelopment outcomes will be recorded thus allowing for better comparison between the diverse treatment protocols in use.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-040

Pragmatic skills and executive functions in children with congenital cytomegalovirus infection and cochlear implants - a comparative cross-sectional study

Ulrika Löfkvist¹, Lena Anmyr², Eva Karltorp²

¹Oslo University, OSLO, Norway

²Karolinska Institutet, STOCKHOLM, Sweden

Congenital cytomegalovirus (cCMV) infection is common and may result in progressive hearing loss or deafness. In our previous study general outcome was explored in a group of children with hearing impairment (HI) caused by cCMV infection with cochlear implants (CI). Around 90 % of children with cCMV infection had severely damaged balance function leading to late on-set of walking (Karltorp et al., 2014). Around 20 % had vision impairment, 15 % were diagnosed with Autism-Spectrum-Disorder and 20 % with ADHD. One clinical observation during assessments was that the majority of children with cCMV infection had problems with executive functioning (EF) like impulse control and attention span while controls with connecin 26 caused HI did not have similar difficulties. Therefore, a new follow-up study was initiated with the main objective of examining EF but also pragmatic skills, non-verbal cognitive ability and language outcome, with a multidisciplinary team approach using standardized tests. Twenty-one children with cCMV infection and CI aged 1:10-18:3 years and eleven controls with cx26 and CI aged 1:0-14:7 years participated. Children with cCMV had statistically significant worse pragmatic skills outcome than controls. However, children with cCMV had age-appropriate vocabulary and non-verbal cognitive skills but showed atypical EF results. Conclusions: children with cCMV

infection are at risk of developing learning difficulties in school due to EF problems and experience difficulties with pragmatic understanding in social interactions. Preliminary results will be presented and in addition clinical implications of the overall outcome will be discussed during the presentation.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-041

Analytical performance of CMV serodiagnosis at birth and in early primary infection

Klaus Hamprecht, Harald Abele, Karl Oliver Kagan, Imma Fischer, Gerhard Jahn

University Hospital of Tuebingen, Institute of Medical Virology, TUEBINGEN, Germany

Background: Diagnosis of CMV primary and recurrent infection in pregnancy is mainly based on serology and in second line on PCR. In the absence of an international CMV-IgG standard for calibration, many commercial manufacturers offer test systems mainly based on EIA systems using recombinant or CMV-AD169-lysate antigens. It is well known, that many IgM-test systems as well as CMV-IgG-avidity test systems may differ drastically in results. In order to investigate the analytical performance of different commercially available test systems, we checked well defined sera from nearly 1300 mothers at birth and about 70 defined sera from early CMV primary infections.

Methods: We perform a combined mother-and infant CMV screening at birth to evaluate the frequency of congenital CMV infection in our institution. In 2011, we had 1150 evaluable mother-infant pairs at birth and defined three serological cohorts: IgGneg/IgMneg (I), IgGpos/IgMneg (II) and IgG pos/IgMpos/IgG avidity high (III). We used test systems from Medac, Diasorin, abbott, Roche, Siemens, and Mikrogen (recomLine blot).

Results: Using this approach we found concordant results ("5" vs reClB) for cohort I (seronegative) in 516/553 cases (93,1%), and discordant results in 37/553 cases with 14 cases of wrong borderline IgM tests. In the latently infected maternal cohort we found in 499/514 cases concordant results (97,1). The discordant 15 cases are also based on false positive IgM test. Concordant results in cohort III were only found in 6/60 cases (10%!). In early primary infection we compared the test systems of Roche and abbott and found also highly divergent results, especially IgM-indices and IgG-avidity.

Conclusions: Our study unequivocally shows, that for any screening purpose in early pregnancy only CMV IgG test systems show reliable analytical performance. The german AWMF guideline for diagnosis of viral infections in pregnancy (2014) therefore recommends a booking sample at the first diagnosis of pregnancy.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-008

CMV-specific T cells through retroviral transduction of gammadelta T cells for adoptive T cell transfer

Clemens Joos, Larissa Martin, Xiaoling Liang, Stefanie Ameres, Andrea Schub, Andreas Moosmann
Helmholtz Zentrum München, Research Unit Gene Vectors, MÜNCHEN, Germany

Human Cytomegalovirus (HCMV) frequently reactivates after hematopoietic stem cell transplantation (HSCT). Standard therapeutic options, however, are not universally effective and have side effects that prohibit prophylaxis and limit their pre-emptive use. Since adoptive transfer of CMV-specific T lymphocytes can protect patients at risk of CMV disease, these cells are key to controlling CMV infection. But if a CMV-seropositive recipient receives HSCs from a CMV-seronegative donor, the latter

cannot provide virus-specific T lymphocytes to prevent reactivation of CMV.

Transferring the T cell receptor (TCR) genes of CMV-specific T cell clones to donor T cells will convey CMV specificity to those cells. If $\alpha\beta$ T cells receive another $\alpha\beta$ TCR, both TCRs may form mixed heterodimers with new and potentially hazardous specificities. This drawback may be obviated by employing donor $\gamma\delta$ T cells to receive a CMV-specific $\alpha\beta$ TCR. Additionally, $\gamma\delta$ T cells can be effectively expanded by approved drugs such as bisphosphonates. It is also believed that $\gamma\delta$ T cells are less likely to cause graft-versus-host disease in an allogeneic recipient.

For use of this application, we established a panel of CMV-specific CD4+ and CD8+ T cell clones targeting 16 distinct epitopes of pp65, IE-1 and UL28 presented by a wide range of MHC I and MHC II allotypes. The α and β chains of twenty-seven specific TCRs were characterised and bicistronically cloned into retroviral vectors. Recognition and lysis of CMV-infected cells of different type by TCR-transduced $\gamma\delta$ and $\alpha\beta$ T cells will be tested. TCRs with optimal virus-specific function in such a transgenic system will be further developed for adoptive immunotherapy.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-009

Functional impairment of CMV-reactive cellular immunity during pregnancy

Anne Rasclé¹, Edith Reuschel², Sascha Barabas¹, Florian Zeman³, Hanna Bendfeldt¹, Ludwig Deml¹, Birgit Seelbach-Goebel²

¹Lophius Biosciences, REGENSBURG, Germany

²University of Regensburg, Hospital of the Barmherzige Brueder, Clinic St. Hedwig, Obstetrics and Gynecology, REGENSBURG, Germany

³University of Regensburg, Center for Clinical Studies, REGENSBURG, Germany

Cytomegalovirus (CMV) is the most common congenital viral infection in developed countries. Mother-to-child transmission can cause severe child disability, such as psychomotor retardation and hearing loss. Intact CMV-specific cell-mediated immunity (CMI) prevents uncontrolled replication in healthy individuals. This study aimed to determine whether CMV-specific CMI is impaired in pregnant women, thus potentially increasing the overall risk of active CMV replication and transmission.

CMV-specific CMI in peripheral blood of 60 pregnant women was determined using T-Track[®] CMV, a novel immune-monitoring IFN- γ ELISpot assay quantifying CMV-reactive effector cells in response to T-activated[®] pp65 and IE-1 proteins. T-Track[®] CMV results were analyzed in relation to CMV-IgG and CMV-IgM serostatus.

CMV-specific CMI was detected in 65% of CMV-seropositive pregnant women. 5% of CMV-IgG seronegative women showed IE-1- but not pp65-reactive cells. The overall number of CMV-reactive cells in pregnant women was significantly lower compared to that of a matched non-pregnant control group ($p < 0.001$). No significant difference in CMV-specific CMI was detected in the course of the three trimesters of pregnancy in CMV-IgG seropositive women. Postpartum (median days postnatal = 123), IE-1- and pp65-specific CMI remained significantly lower than in the non-pregnant control group ($p < 0.001$ and 0.0032, respectively). Functional analysis of CMV-reactive immune cells using T-Track[®] CMV therefore suggests a systemic down-regulation of CMV-specific CMI in pregnant women. Further studies are needed to investigate whether this may be indicative of a higher susceptibility to CMV reactivation or transmission.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-012

Evaluation of cytomegalovirus-specific cell-mediated immunity in heart transplant recipients by two different Enzyme-linked ImmunoSPOT (EliSpot) assays

Angela Chiereghin¹, Giulia Piccirilli², Liliana Gabrielli², Gabriele Turello², Diego Squarzonì², Laura Borgese³, Luciano Potena³, Tiziana Lazzarotto²

¹Policlinico S. Orsola-Malpighi, University of Bologna, O.U. of Microbiology, Laboratory of Virology, BOLOGNA, Italy

²O.U. of Microbiology, Policlinico S. Orsola-Malpighi, University of Bologna, BOLOGNA, Italy

³Cardiovascular Department, Policlinico S. Orsola-Malpighi, University of Bologna, BOLOGNA, Italy

The cytomegalovirus (CMV)-specific cell-mediated immunity (CMI) has been identified as an essential host factor for controlling CMV infection and currently there is a variety of CMV-specific T-cell assays available.

This study evaluated the performances of two commercial EliSpot assays - EliSpot Interferon- γ Basis Kit (GenID GmbH) and T-SPOT.CMV test (Oxford Immunotect Ltd) - for the evaluation of CMV-specific CMI.

Twenty serial blood specimens collected from seven heart transplant recipients during the post-transplant period (min-max, 1-6 months) were prospectively processed by both assays. All patients were CMV-seropositive at the time of transplant; five patients received anti-CMV prophylaxis (3 months) and two were managed pre-emptively. The immunological monitoring was performed during the first biopsy and at 1, 3 and 6 months post-transplant. CMV-DNAemia monitoring was performed by real-time PCR (ELITechGroup).

A positive response to at least one out of the two CMV-specific antigen stimulations (IE1 and pp65-UL83) identified an EliSpot positive result and therefore a patient with detectable CMV-specific CMI. Among the 20 samples, 18 (90%; n=6 patients) resulted positive and 2 (10%; n=1 patient) resulted negative by both assays. In 12/18 (66.7%) samples, CMV-IE1-specific CMI was detected only by T-SPOT.CMV test. Among the six patients with detectable CMV-specific CMI, 4 (66.6%) developed an asymptomatic CMV infection with low viral load (median 5,404 copies/mL; min-max 1,543-8,111). The patient without CMV-specific CMI developed an asymptomatic infection with a higher viral load (peak CMV-DNAemia 18,420 copies/mL) and received antiviral therapy.

In this preliminary phase, the two immunological assays showed an agreement equal to 100%, although the CMV-IE1 antigen provided in T-SPOT.CMV test was more immunogenic than the one used in EliSpot Interferon- γ Basis Kit. Both assays proved to be support tools to identify the patient who would benefit most from pre-emptive interventions. The performances of these assays in cryopreserved samples will be also assessed.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
CD-PP-002

Trends in valganciclovir use among infants with congenital cytomegalovirus infection in the United States, 2009-2014

Jessica Leung, Tatiana Lanzieri, Sheila Dollard, Winnie Chung, ThuyQuynh Do, Manisha Patel, Scott Grosse

Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases, ATLANTA, GA, United States of America

Introduction: Data from clinical trials have shown that valganciclovir treatment may improve hearing and neurodevelopmental outcomes in infants with congenital cytomegalovirus (cCMV) infection who are symptomatic at birth. Using a large health insurance claims database, we examined trends in valganciclovir use among infants with cCMV.

Methods: We analyzed 2009-2014 medical claims from Truven Health MarketScan[®] Commercial Claims and Encounters and Medicaid databases. We defined infants as enrollees aged <1 year with a newborn code in the first claim, and cCMV diagnosis as CMV or cCMV code within 45 days of the newborn code. Among infants with cCMV, we assessed the frequency of 1) cCMV-associated conditions (i.e. chorioretinitis, hepatomegaly, splenomegaly, jaundice, low birth weight, microcephaly, petechiae, or

thrombocytopenia) within 45 days of the newborn code, 2) hearing loss within 180 days of the newborn code, and 3) outpatient drug claims for valganciclovir.

Results: Among 1,029,393 infants with commercial insurance, 174 (0.02%) had a cCMV diagnosis; of which 100 (57%) had ≥ 1 CMV-associated condition and 17 (10%) had hearing loss. Twenty-six (15%) infants with cCMV had valganciclovir claims, among which 21 (81%) had ≥ 1 cCMV-associated condition and/or hearing loss. Among 1,093,008 infants with Medicaid insurance, 268 (0.03%) had a cCMV diagnosis; of which 197 (74%) had ≥ 1 cCMV-associated condition and 14 (5%) had hearing loss. Forty-six (17%) infants with cCMV had valganciclovir claims, among which 41 (89%) had ≥ 1 cCMV-associated condition and/or hearing loss. During 2009-2014, the proportion of infants with cCMV who had valganciclovir claims increased from 0% to 19% among infants with commercial insurance and from 6% to 42% among infants with Medicaid insurance.

Conclusions: During 2009-2014, there was a strong upward trend in valganciclovir claims among infants with health insurance who had a cCMV diagnosis, the majority of whom had a cCMV-associated condition and/or hearing loss.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-003

A third component of the human cytomegalovirus terminase complex is involved in letermovir resistance

Sunwen Chou

Oregon Health & Science University, PORTLAND, United States of America

Letermovir is a human cytomegalovirus (CMV) terminase inhibitor antiviral compound that was clinically effective in a Phase III prevention trial. In vitro studies have shown that viral mutations conferring letermovir resistance map primarily to the UL56 component of the terminase complex. Uncommonly selected in vitro, UL89 component mutations N320H and D344E confer a borderline letermovir resistance phenotype. After serial culture of wild type CMV under letermovir, mutation has now been observed in a third component of the terminase complex in 2 experiments at passages 10 and 25, both resulting in amino acid substitution P91S in gene UL51 and adding to a pre-existing UL56 mutation. Recombinant phenotyping confirmed that P91S alone conferred 2.4-fold letermovir resistance over baseline, and when combined with UL56 mutation S229F or R369M, multiplied the level of resistance conferred by those mutations by 3- to 6-fold. Similarly a combination of UL56 mutations S229F, L254F and L257I selected in the same experiment conferred 50-fold increased letermovir resistance, but 250-fold when combined with UL51 P91S. The P91S mutant was not perceptibly growth impaired in susceptibility assays. Although pUL51 is essential and believed to affect localization of the terminase complex by interaction with pUL56, its biological significance is not well understood. Letermovir resistance mutations mapping to 3 separate genes and their multiplier effect on the level of resistance suggests that the mutated residues of the various terminase components converge on a drug-binding site or exert their effect through more remote conformational effects. Full genotypic screening for letermovir resistance now requires consideration of UL51 mutation.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-005

Inhibitory effect of triclin on CCL2-CCR2 dependent human cytomegalovirus replication

Tsugiyu Murayama, Hidetaka Sadanari, Masaya Takemoto, Keiko Matsubara, Tohru Daikoku, Faculty of Pharmaceutical Sciences, Hokuriku University, Department of Microbiology and Immunology,

KANAZAWA, Japan

Human cytomegalovirus (HCMV) infection enhances the expression of the CXC chemokine CXCL8, and the CC chemokines CCL2 and CCL5. HCMV infection is presumed to contribute to atherosclerosis, where chemokines may have a pathogenic role. Elevated levels of CCL2 are observed in atherosclerotic plaques, where macrophages with the expression of a specific receptor for CCL2, CCR2, abundantly infiltrate. Thus, HCMV and CCL2 may cooperatively contribute to atherosclerosis. Our recent study revealed that tricetin (4',5,7-trihydroxy-3',5'-dimethoxyflavone) has anti-HCMV activity in a human embryonic lung fibroblast cells (HEL). In the present study, we revealed that HCMV-induced CCL2 expression further augments HCMV infection and that tricetin exerts its anti-HCMV activity by targeting CCL2.

HCMV Towne strain was propagated in HEL cells. Infectious virus production was titrated by using a plaque assay. The tricetin compound used was synthetic. siRNAs targeting CCL2 was purchased from a company. Proteins were detected by Western blot analysis. Gene expressions were detected using the reverse transcription quantitative real-time PCR analysis.

HCMV infection induced CCL2 and CCR2 expression at the mRNA and the protein levels in HEL cells. CCL2 siRNA treatment reduced HCMV virion production. We further observed that CCL2 siRNA reduced the expression of HCMV IE and UL54 genes in a dose-dependent manner. Thus, HCMV infection can activate the CCL2-CCR2 interactions to further enhance HCMV infection and/or replication. Next, we observed that HCMV-induced CCL2 mRNA and protein expression was inhibited by tricetin and exhibited inhibitory activities against HCMV replication. Thus, tricetin exerts its anti-HCMV activities at least partly by inhibiting the expression of a CCL2, which can support HCMV infection and/or replication.

These results suggest that tricetin is a novel compound with potential anti-HCMV activity and that CCL2/CCR2 interactions are associated with HCMV replication.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-016

Usefulness of Viral Load Measurement and Therapeutic Drug Monitoring in the Treatment of Infants with Cytomegalovirus Disease

Hiroyuki Moriuchi¹, Moriuchi Masako¹, Sato Kayoko², Kitahara Takashi², Sasaki Hitoshi²

¹Nagasaki University Graduate School of Biomedical Sciences, Department of Pediatrics, NAGASAKI, Japan

²Nagasaki University Hospital, Department of Hospital Pharmacy, NAGASAKI, Japan

Background: The pharmacokinetics of anti-CMV agents ganciclovir and its prodrug valganciclovir has not been fully investigated in infants, especially very-low-birth-weight infants, and appears to be very unstable. Moreover, the optimal pharmacokinetics has not been determined. Side effects are common and sometimes indistinguishable from clinical manifestations caused by CMV itself, making it difficult to evaluate therapeutic efficacy and safety.

Objective: To optimize antiviral treatment of infants with active CMV disease by regular viral load (VL) measurement and therapeutic drug monitoring (TDM).

Methods: Since September 2015, infants with symptomatic CMV infections, either congenital or acquired, had been enrolled in Nagasaki University Hospital or collaborating medical institutes. VLs in the whole blood were measured by real-time PCR weekly during therapeutic period. Plasma ganciclovir concentrations were determined by liquid scintigraphy/tandem mass screening. Peripheral blood was drawn weekly to monitor side effects during therapeutic period.

Results: TDM was performed 76 times for 41 cases. We tentatively defined targeted AUC_{0-12} as 20-50 $mg \times h/L$ and targeted C_{max} as $>5 mg/L$; however, AUC_{0-12} were often below or above planned, and C_{max} values were below 5 mg/L in 34 times. Although we adjusted dose size and dosing interval according to their renal function, it was often difficult to optimize them. In some patients who developed cytopenia or liver dysfunction, VLs and drug concentrations along with chronological observation of clinical manifestations were the basis for the judgment to differentiate side effects from viral disease activities. A patient with active retinal disease needed approximately 150% of the regular dose size to

achieve reasonably high levels of C_{max} and AUC₀₋₁₂, followed by improvement of retinal lesion.

Conclusions: The pharmacokinetics of ganciclovir or valganciclovir was difficult to predict in infants who are prone to their side effects. Regular VL measurement and TDM are needed for effective and safe treatment for them.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-018

Nanobodies targeting oncomodulatory CMV-encoded chemokine receptors as potential diagnostics and therapeutics

Martine Smit, Raimond Heukers, Tian Shu Fan, Raymond De Wit, Rob Leurs, Marco Siderius, Henry Vischer

Vrije Universiteit Amsterdam, Dept of Medicinal Chemistry, AMSTERDAM, Netherlands

Cytomegalovirus rewires cellular signaling after viral infection through expression of viral G protein-coupled receptors (GPCRs). These viral GPCRs show highest homology to chemokine receptors, which are known to regulate the immune system but are also involved in the development of cancer. We have shown that several viral GPCRs, including the CMV-encoded chemokine receptor US28, signal in a constitutive manner and hijack proliferative signaling pathways (Vischer et al Nat Rev Drug Disc 2014). US28 stimulates cell proliferation and enhances tumour formation in an orthotopic xenograft model and is expressed in tumour specimen of glioblastoma patients. Moreover, US28 induces neoplasia in the intestine of US28 expressing transgenic mice via activation. Interestingly, US28 is one of the few viral genes that are expressed during latency. Recently, we have identified llama-derived antibody fragments, nanobodies, which effectively bind and modulate (oncogenic) signaling of US28. Moreover, nanobodies targeting US28 impair tumor progression of glioblastoma in vivo. Hence, the US28-specific nanobodies hold potential as CMV-targeting therapeutics, diagnostics and research tools, to further substantiate a role for US28 in latency and CMV-associated pathologies.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-029

Experience of a translational research platform for the evaluation of HCMV drug-resistance in Belgium

Graciela Andrei, Sarah Gillemot, Robert Snoeck
Rega Institute - KU Leuven, LEUVEN, Belgium

Drug-resistance in human cytomegalovirus (HCMV) is virtually not observed in immunocompetent individuals but it is a well-recognized problem among different populations of immunocompromised patients. Therefore, in 2009 a Reference and Service Center, **RegaVir** [Research Group for Antiviral Resistance, (www.regavir.org)], for the diagnosis and typing of drug-resistant herpesviruses was established in Belgium.

Genotyping [PCR amplification of the HCMV genes UL97 (protein kinase, PK) and UL54 (DNA polymerase, DNA pol followed by capillary (Sanger) sequencing] are used to diagnose resistance to the approved anti-HCMV drugs ganciclovir, cidofovir and foscavir. Today, in the context of the RegaVir

platform, we have analyzed 628 clinical samples recovered from patients that had refractory HCMV disease. A total of 536 out of 628 samples could be genotyped, with 370 showing a wild-type genotype. Mutations known to be linked to drug-resistance were found in 27.6% of the genotyped samples: 105 samples had mutations in the PK, 24 in the DNA pol and 12 samples bore mutations both in the PK and DP genes. Novel mutations of unknown significance in the PK and/or DP genes were found in 35 samples.

Our data showed a) the usefulness of rapid HCMV genotyping for the adjustment of antiviral therapy, b) emergence of multiple drug-resistance due to infection with a virus having a single mutation conferring resistance to ganciclovir, cidofovir and foscavir (e.g. DNA pol mutations A834P and del 981-982) or caused by co-infection with viruses having distinct genotypes, c) compartmentalization of drug-resistant HCMV, d) advantage of next generation sequencing (NGS) for detecting minor populations of drug-resistant viruses, e) emergence of resistance to investigational anti-HCMV drugs (e.g. maribavir), f) urgent need for novel anti-HCMV agents to reduce morbidity and mortality caused by drug-resistant HCMV.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-030

Combined biochemical and in silico-structural analyses of cytomegalovirus kinase pUL97 to specify the targeting of inhibitory small molecules

Manfred Marschall¹, Mirjam Steingruber¹, Hanife Bahsi¹, Stuart Hamilton², William Rawlinson², Eileen Socher³, Heinrich Sticht³, László Örfi⁴, Stefan Strobl⁵, Sunwen Chou⁶, Joachim Haupt⁷, Corina Hutterer¹

¹Institute for Clinical and Molecular Virology, Friedrich-Alexander University of Erlangen-Nürnberg, ERLANGEN, Germany

²Serology and Virology Division, SEALS Prince of Wales Hospital, University NSW, SYDNEY, Australia

³Division of Bioinformatics, Institute of Biochemistry, FAU, ERLANGEN, Germany

⁴Semmelweis University, Department of Pharmaceutical Chemistry, BUDAPEST, Hungary

⁵BioNTech Small Molecules GmbH, MARTINSRIED, Germany

⁶Division of Infectious Diseases, Oregon Health and Science University, PORTLAND, United States of America

⁷Biotechnology Center, Technische Universität Dresden, DRESDEN, Germany

Human cytomegalovirus (HCMV) is a worldwide distributed human pathogen that can be associated with serious, sometimes life-threatening disease. All currently applied drugs face problems of toxicity-related side effects and drug resistance, so that novel antiviral targets and drug candidates are continuously investigated. The viral protein kinase pUL97 represents a validated and highly interesting target of antiviral kinase inhibitors. Our ongoing biochemical analysis of pUL97 has been focused on its modular domain structure, its interaction properties and binding motifs (in particular for pUL97-cyclin interaction) as well as regulatory factors of kinase activity. As a multi-ligand-binding, multi-regulatory protein kinase, pUL97 provides ample opportunities for molecular interference and the targeting of inhibitory small molecules. We are utilizing bioinformatic tools to optimize *in silico*-structural analysis of inhibitor binding and to define chemical modifications intended to specify the pUL97 targeting. Novel data for the quinazoline class inhibitors Vi7392 and Vi7453 stressed our earlier findings that quinazolines exert a high antiviral efficacy without inducing measurable *in vitro* drug resistance. This prompted us to use the quinazoline core structure to gain deeper insight into the mode of inhibitor-kinase interaction. We demonstrate that (i) novel quinazolines are highly active against HCMV laboratory and clinically relevant strains, (ii) antiviral activity is not cell-type specific and is also detectable in a placental explant tissue model, (iii) pUL97 represents a target of the compounds' antiviral activity and (v) drug docking simulation helps to understand resistance-free pUL97 binding properties. In particular, reduced frequency of drug resistance may result from a tight mode of quinazoline-pUL97 docking or, alternatively, by a dual selective mode of kinase inhibition involving secondary cellular targets. To this aspect, novel data of a kinome-scan profiling analysis will be presented. Combined, the approach may open new prospects for using information on drug-target interaction for rational design of antivirals.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-032

Cytomegalovirus infection and antiviral drug resistance in hematopoietic stem cell transplant recipients

Giulia Piccirilli¹, Mariarosaria Sessa², Francesca Bonifazi², Giuseppe Bandini², Angela Chiereghin¹, Liliana Gabrielli¹, Gabriele Turello¹, Diego Squarzone¹, Gian Maria Rossolini³, Riccardo Saccardi⁴, Tiziana Lazzarotto¹

¹O.U. of Microbiology, Policlinico S. Orsola-Malpighi, University of Bologna, BOLOGNA, Italy

²O.U. of Hematology and Oncology, St. Orsola-Malpighi, University Hospital, BOLOGNA, Italy

³SOD Microbiologia, A.O.U. Careggi, Università di Firenze, FIRENZE, Italy

⁴SOD Terapie Cellulari e Medicina Trasmfusionale, A.O.U. Careggi, FIRENZE, Italy

Cytomegalovirus (CMV) remains an important complication after allogeneic hematopoietic stem cell transplant (allo-HSCT). The widespread use of CMV antiviral therapy has led to the development of drug resistance.

The study describes CMV drug resistance in adult allo-HSCT recipients monitored for CMV infection and pre-emptively managed with valganciclovir (VGCV).

From November 2014 to October 2016, 92 HSTC recipients were monitored for CMV infection by quantitative real-time PCR (ELITechGroup, Italy) on whole blood samples. If CMV drug resistance was suspected (CMV-DNAemia persisted for >3 weeks after antiviral treatment), sequence analysis of UL97 and UL54 genes was performed to identify the most common mutations associated with CMV drug resistance.

Median follow-up duration for the 92 allo-HSTC recipients in our hospital was 241 days (range: 20-533 days). The 64.1% (59/92) of patients developed active CMV infection (median time for first episode: 30 days post-transplant, range: 7-74 days) and the 64.4% (38/59) received VGCV therapy. CMV drug resistance was suspected in 10 cases and confirmed in 3. Single mutations in UL97 gene associated with GCV resistance was found in all 3 cases: A594V (n=2) and L595S (n=1). For these patients antiviral treatment was shifted to foscarnet (FOS) that led to undetectable CMV DNAemia in all cases. No patient developed CMV disease. Moreover, an allo-HSTC recipient from another Italian hospital was evaluated for suspected CMV drug resistance (DNAemia persisted for 113 days during VGCV therapy then switched to FOS and then cidofovir). The A594V (UL97 gene) mutation was detected and FOS was continued followed by cidofovir. After an initial decrease, CMV load increased again two months later. An additional mutation (A834P) in UL54 gene, associated with GCV, FOS and cidofovir resistance emerged. The management of the patient is still under evaluation.

CMV drug resistance detection is a relevant tool to guide the choice of antiviral therapy.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-033

Effects of letermovir and Bay 38-4766 on human cytomegalovirus DNA cleavage and packaging

Anne Sauer, Michael McVoy

Virginia Commonwealth University, RICHMOND, United States of America

Letermovir is a human cytomegalovirus (HCMV) antiviral currently in phase 3 testing. It combines high potency ($EC_{50} = 5$ nM) with low cytotoxicity. Letermovir is thought to inhibit the HCMV terminase, a three subunit complex of UL56, UL89, and UL51 that translocates concatemeric viral DNA into preformed capsids and cleaves the DNA to liberate encapsidated genome monomers. Bay 38-4766 and 2-bromo-

5,6-dichloro-1-β-D-ribofuranosyl benzimidazole riboside (BDCRB) are structurally different but also inhibit HCMV by targeting terminase. Letermovir resistance maps to UL56, while Bay 38-4766 resistance maps to UL89, and BDCRB resistance maps to both UL56 and UL89. Bay 38-4766 and BDCRB impair the formation of genome monomers, but BDCRB also induces formation of a novel HCMV DNA species designated monomer-plus (M+) because its apparent 270-kb size is slightly larger than 235-kb monomers. Bay 38-4766 also induces M+ formation, but only when a Bay 38-4766-resistant mutant is used. In previous studies we hypothesized that M+ is comprised of two short genome segments flanking one long segment and arise when BDCRB or Bay 38-4766 impairs terminase cleavage site recognition without blocking DNA translocation, thus causing an extra short segment to be packaged prior to cleavage at the next short-long junction. To determine if letermovir inhibits monomer formation and/or induces M+, wild type HCMV-infected cells were treated with increasing concentrations of letermovir, Bay 38-4766, or BDCRB. Cell-associated DNA species were then separated by field-inversion gel electrophoresis and viral species detected by Southern hybridization. As expected, BDCRB inhibited monomer formation and induced M+. Letermovir and Bay 38-4766 also reduced or eliminated monomer formation, but no evidence for M+ was observed. Evaluation of letermovir- and Bay 38-4766-resistant mutants is in progress. These results indicate that the mechanisms of action for these terminase inhibitors have both common and distinct aspects that will require further study to elucidate.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-037

Brincidofovir resistance during rescue therapy in French transplant recipients

Sophie Alain¹, Vanessa Escuret², Pascale Boissonnat³, Christine Zandotti⁴, Tristan Legris⁴, Sebastien Hantz¹, Eliza Munteanu¹, Melissa Gomes-Mayeras¹, Christine Archimbaut⁵, Tatiana Galperine⁶, Marianne Leruez-Ville⁷, Pierre Frange⁷, Alexandra Ducancelle⁸, Alain Coaquette⁹, Annie Dewilde⁶, Arnaud Lionet⁶, Jean-Christophe Plantier¹⁰, study group BCV resistance¹¹

¹National Reference Center for Cytomegaloviruses, CHU Limoges, Virology, LIMOGES, France

²Centre de Biologie et de Pathologie Nord HCL Lyon, Virology, LYON, France

³Hopital Louis Pradel, Hospices Civils de Lyon, Heart transplantation, BRON, France

⁴CHU Hopital de la Timone, Virologie, MARSEILLE, France

⁵CHU Clermont Ferrand, Virology, CLERMONT-FERRAND, France

⁶CHU Lille, Infectious diseases, LILLE, France

⁷CHU Necker-enfants Malades, Virology, PARIS, France

⁸CHU Angers, Virology, ANGERS, France

⁹CHU de Besançon, Virology, BESANÇON, France

¹⁰CHU de Rouen, Virology, ROUEN, France

¹¹Participants, ALL SITES, France

Brincidofovir (BCV) is an oral lipid acyclic nucleoside phosphonate converted intracellularly in cidofovir. As it is not concentrated in the renal proximal tubule, nephrotoxicity is unlikely. This drug proved efficient against CMV in a phase II study in stem-cell recipients and was released in France during 2014-2015 for resistant CMV infection in transplant recipients, within a name patient program (NPP). As a reference center we surveyed antiviral-resistance, and report our experience in 10 BCV-treated patients. We included 2 stem cell paediatric allograft recipients (1 refractory viremia and 1 non-resistant CMV colitis) and 6 kidney and 2 heart adult recipients (3 CMV disease, 2 acute rejection, 3 refractory CMV infection), 6/8 with a low mitogen response by Quantiferon-ÔCMV. Absence of UL54 cidofovir-resistance mutation before BCV was further confirmed by NGS analysis (Ion Proton technology). 6 patients were resistant to Ganciclovir (GCV) alone by UL97 mutation. Paediatric patients received 2mk/kg twice a week (t.i.w.), adults 200 or 100mg t.i.w. Anti-CMV hyperimmune-globulins were co-administered to 4 adult patients. Mean viral load at initiation of treatment was 4,2 log copies/mL (2,9-5,2). The 2 paediatric patients did not responded to treatment with stable or increased viral load and majoration of diarrhea within 3 weeks BCV for one patient, but no resistance. In adult patients after initial decrease of viral load, 5/8 did not responded to BCV. UL54 resistance emerged in 3/5 patients (L545S, L513D, F412L). NGS genotyping showed the presence of multiple strains in resistant patients and resistance selection as early as day 34 of BCV in one patient. Acute renal failure, microangiopathy, or digestive toxicity lead to

low doses or treatment interruption.

Conclusion: Initial efficacy in most patients shows BCV antiviral efficacy but digestive troubles and low doses may lead to early resistance in these patients with a very low CD8 global response.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-013

Expression of Simian Immunodeficiency Virus (SIV) Antigens by Bacterial Artificial Chromosome (BAC)-Derived Infectious Cynomolgus Macaque CMV (CyCMV)

Justen Hoffman Russell¹, Angie Marsh¹, David Willer¹, Ambagala Aruna², Meika Richmond³, Fatima Csordas¹, Jacqueline Chan¹, Richard Pilon⁴, Paul Sandstrom⁵, Kelly MacDonald³

¹University of Toronto, Immunology, TORONTO, Canada

²National Centre for Foreign Animal Disease, WINNIPEG, Canada

³University of Manitoba, Internal Medicine, WINNIPEG, Canada

⁴Public Health Agency of Canada, Centre for Biosecurity, OTTAWA, Canada

⁵JC Wilt Infectious Diseases Research Centre, National HIV and Retrovirology Lab, WINNIPEG, Canada

Introduction: Though closely related to rhesus macaques, cynomolgus macaques respond differently to a number of infectious diseases, including HIV/SIV where disease progression closely mimics human disease. However the cynomolgus model is currently underutilized in biomedical research, in part due to underdevelopment of reagents and molecular tools. CMV has recently emerged as a promising vaccine vector. We add to the available resources a full length BAC based on the CyCMV Mauritius strain and utilize this new tool to express SIV transgenes throughout CMV infection.

Methods: The BAC vector (pWC155) was integrated by sequential recombination steps between CyCMV Mauritius open reading frames (ORFs) CyO19 and CyO20, facilitating bacterial replication of the circularized viral form. Two codon-optimized SIVmac239 transgenes were designed on EF-1 α -BGHpA expression cassettes: 490aa Gag-Pol fusion (GPF), and 1147aa Nef-Tat-Rev fusion (NTR). Transgenes were integrated by BAC recombination between ORFs CyUS3 and Cy215 and expression observed in reconstituted viral infection of Telo-RF cells.

Results: CyCMV Mauritius BAC reconstituted as a virus with no change in growth kinetics beyond standard error in cynomolgus macaque, rhesus macaque, or human cell lines despite unavoidable attenuation. BAC integration resulted in 789 in-frame Polymorphisms (682 SNP, 107 Indel) altering 64/290 known ORFs (36 with \leq 3aa changed). The majority of polymorphisms occurred in strain-specific or highly variable transmembrane proteins. However single aa changes occurred in notable genes CyUL47, CyUL87 and CyUL102, while CyUL146 and CyUL147 (CXC-chemokine like), and CyUL128/CyUL130/CyUL131 (cell tropism factors) were substantially altered. Following transgene integration robust expression of both NTR and GPF proteins was observed.

Conclusion: CyCMV Mauritius BAC, a stable, tractable, infectious equivalent of HCMV in the cynomolgus model, expressed SIV transgenes throughout viral infection. As a molecular tool, CyCMV Mauritius BAC expands CMV vaccine vector studies to the cynomolgus model and will facilitate better utilization of cynomolgus macaques for CMV research.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-019

Construction of replication competent high capacity vectors based on murine cytomegalovirus

Zsolt Ruzsics, André Riedl, Denisa Bojková, Patrick König, Sebastian Naumann, Peter Zimmermann
University Medical Center Freiburg, Institute of Virology, FREIBURG, Germany

We investigated the basic characteristics of a new murine cytomegalovirus (MCMV) vector platform. Using BAC technology, we constructed a replication competent recombinant MCMV vectors lacking up to 24% of the wildtype genome and carry combined deletions of the five gene blocks m01-m017, m106-m109, m129-m141, m144-m158, and m159-m170. While deletion mutants lacking 24-18% of wildtype genome were attenuated in fibroblasts, smaller deletions (up to 16%) grown like wildtype virus. By means of a new methodology we inserted large genomic DNA segments (33 and 42 kbp in size) as stuffer together with reporter genes into an m01-m017, m106-m109, m144-m158, and m159-m170 deficient vector with a potential cloning capacity of 46kbp (Q4). Surprisingly, the insertion of both foreign DNAs rescued the growth phenotype of Q4 and the large inserts were stably maintained during serial passages *in vitro*. Using reporter gene expressing recombinant MCMVs, we could successfully transduced, beside of mouse cell lines, non-rodent mammalian cells with human, monkey, bovine, bat origin, and even non-mammalian cell lines derived from chicken. We also tested toxicity of the MCMV mediated gene transfer and investigated the abortive replication cycle of the MCMV-vectors in human cells upon transduction.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-010

CMV screening during pregnancy in Belgium: the gap between theory and practice.

Catharina Mathei¹, Edith Gellens²

¹KULeuven, LEUVEN, Belgium

²Private Practice, WILSELE, Belgium

In Belgium CMV screening in pregnant women is common practice despite international as well as national guidelines not recommending so. Screening of pregnant women could lead to anxiety, an increased number of amniocentesis and therefore of miscarriages, and an unjustified number of termination of pregnancies. In this qualitative study we investigated why there is this gap between theory and practice.

Forty-six experts (gynaecologists and general practitioners (GPs)) involved in pregnancy management participated in a two-round argument Delphi approach to explore the arguments that would plead for or against CMV screening in pregnant women.

General practitioners tend to follow the guidelines whereas the majority of the gynaecologists do screen for CMV. The main argument of the latter group is that in case of a positive test echocardiographic follow-up and amniocentesis can be provided which in their opinion has in most cases a reassuring effect. For gynaecologists medico-legal issues were also an argument pro screening. In contrast, GPs perceive this follow-up as a source of concern and distress.

Lack of time and/or knowledge prevents doctors from engaging in informed shared decision making. Few doctors agree that patients should be informed about abortion being the only option in case of a congenital CMV infection nor do they regard the option of an abortion as an argument pro offering screening. According to the experts all pregnant women should be educated about preventive measures regardless of their CMV serology and thus not yields an argument pro or con screening.

In conclusion, opinions of general practitioners and gynaecologists regarding CMV screening differ importantly. Pregnant women are confronted with opposing views, which is obviously very confusing. Efforts should be made to strike the balance between GPs and gynaecologists and tools should be developed to inform the patient and to support the process of shared decision making.

460 - Poster Presentation Track C & D

Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
CD-PP-015

Human cytomegalovirus alters expression of Wnt receptor ROR2 and migration of infected trophoblasts in an autocrine manner

Wendy Van Zuijlen¹, William Paull², Caroline Ford³, William Rawlinson¹

¹Serology and Virology Division, SEALS Microbiology, Prince of Wales Hospital, School of Medical Sciences, University of New South Wales, SYDNEY, Australia

²School of Medical Sciences, University of New South Wales, SYDNEY, Australia

³Metastasis Research Group, Prince of Wales Clinical School, UNSW, SYDNEY, Australia

Human cytomegalovirus (CMV) is the leading infectious cause of congenital malformation in the developed world. Primary maternal CMV infection, reactivation, or infection with a different viral strain may cause adverse pregnancy outcomes including sensorineural hearing loss and mental disability in infants infected *in utero*. Placental infection may also indirectly cause injury to the fetus, possibly via impairing placental development and function. New approaches to disease prevention are urgently needed, as current antivirals are not recommended during pregnancy, due to their toxicity and/or limited efficacy. A better understanding of the molecular mechanisms of CMV infection of the placenta is essential for therapeutic innovations to decrease the prevalence and societal impact of congenital CMV. Our previous findings indicate CMV controls the expression of the Wnt5a-binding tyrosine kinase receptor ROR2 to alter placental cell motility, which could lead to abnormal placental development in congenital CMV disease (van Zuylen et al. J Virol. 2015).

We now show CMV specifically inhibits Wnt5a-mediated migration of infected trophoblasts, but not migration of surrounding uninfected cells. Utilising supernatant from CMV-infected trophoblasts, we also show that this inhibition and ROR2 alteration is not dependent on a soluble factor. Furthermore, we show that both viable laboratory CMV strain AD169 and clinical CMV strain Merlin, but not UV-inactivated CMV inhibits Wnt5a-mediated trophoblast motility, indicating *de novo* viral gene expression is required. Taken together, our novel findings suggest that human CMV alters ROR2 expression, and thereby migration of infected trophoblasts in an autocrine manner. Inhibition of this autocrine signalling may be a specific target for therapeutic intervention for CMV-induced placental damage and consequent fetal damage in congenital CMV infections.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
CD-PP-017

Prevention of congenital CMV through hygiene-based behavioural interventions: A review of the literature and gap analysis

Christine Jones¹, Victoria Barker², Anna Calvert³, Caroline Star⁴, Sharon Wood⁴, Suzanne Luck⁵, Asma Khalil³, Paul Griffiths⁵, Paul Heath³, Tushna Vandrevala²

¹University of Southampton, SOUTHAMPTON, United Kingdom

²Kingston University, KINGSTON UPON THAMES, United Kingdom

³St George's, University of London, LONDON, United Kingdom

⁴CMV Action, LONDON, United Kingdom

⁵University College London, LONDON, United Kingdom

Congenital CMV is the most common non-genetic cause of sensorineural hearing loss in childhood and an important cause of neuro-disability. There is no licensed CMV vaccine and no antenatal treatment of congenital CMV that can currently be recommended in clinical practice. Therefore, reduction of the risk of acquisition of CMV infection in pregnancy by avoidance of contact urine and saliva of young children, who represent the most common source of infection in pregnancy women, represents an appealing strategy. Despite this being identified as potentially one of the most important preventative strategies, information about risk reduction measures are not routinely given to pregnant women in most countries. We aimed to review the evidence for hygiene based educational interventions in pregnancy, synthesizing data in the published literature, identifying ongoing studies and ascertaining research gaps

for prioritization.

There are five published studies from the United States of America (n=3), France (n=1) and Italy (n=1). These studies show that preventative measures are acceptable to pregnant women, can impact on maternal behaviour and have the potential to reduce CMV infection rates in pregnancy. They are however limited by several factors including small sample size, non-randomised trial design and interventions which are beyond the scope of routine clinical practice.

An intervention is still required that can be applied in a routine clinical setting, which does not necessitate significant time commitment in busy antenatal clinics, but can effectively change behavior and reduce the risk of CMV infection and thus prevent congenital CMV. Behavioural change is difficult to bring out and to sustain, however pregnant women are a highly motivated group to protect the health and well-being of their unborn child. The consequences of inaction are significant and therefore the research community should continue to prioritise research to prevent congenital CMV.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-001

Enforced OX40 stimulation empowers booster vaccines to stimulate protective CD4⁺ and CD8⁺ T cell responses against cytomegalovirus infection

Eleni Panagioti¹, Louis Boon², Ramon Arens³, Sjoerd Van der Burg³

¹LUMC, Medical Oncology, LEIDEN, Netherlands

²BIOCEROS, NL, UTRECHT, Netherlands

³Leiden University Medical Center, Immunohematology and Blood Transfusion, LEIDEN, Netherlands

There is an imperative need for effective preventive vaccines against human cytomegalovirus (HCMV) as it poses a significant threat to the immunologically immature, causing congenital disease, and to the immune compromised including transplant recipients. In this study we examined the efficacy of synthetic long peptides (SLPs) as a CD4⁺ and CD8⁺ T cell-eliciting preventive vaccine approach against mouse CMV (MCMV) infection. Immunocompetent C57BL/6 mice were vaccinated in a prime-boost vaccination regimen with various MHC class I and II-restricted peptide epitopes of MCMV-encoded antigens, and the use of agonistic OX40 antibodies to enhance vaccine efficacy was explored. Costimulation via OX40 resulted in superior MCMV-specific CD4⁺ as CD8⁺ T cell responses but only when applied during booster SLP vaccination. A combination of a SLP vaccine with both MHC class I and II epitopes plus OX40 activation during booster vaccination resulted in strong and polyfunctional (i.e., IFN- γ ⁺, TNF⁺, IL-2⁺) CD4⁺ and CD8⁺ T cell responses that were even higher in magnitude when compared to those induced by the virus. Vaccination with a mixture of MHC class II SLPs and OX40 agonistic antibodies already displayed protection against lytic MCMV infection but the best containment of virus dissemination was achieved with the combination of both MHC class I and II SLPs and OX40 costimulation. Our results show that T cell-mediated protection can be a fundamental component in the design of vaccines against persistent viral infections.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-004

Developing fully human monoclonal antibodies as candidate therapeutics for HCMV infection

Jie Ying Jacklyn Neo¹, Vojislav Jovanovic¹, Brendon Hanson², Paul A. MacAry¹

¹National University of Singapore, SINGAPORE, Singapore

²Defence Medical and Environmental Research Institute, DSO National Laboratories, SINGAPORE, Singapore

Treatment options for HCMV infection remain limited despite intensive efforts from global pharma. The four antiviral drugs currently licensed for treatment of HCMV infection exhibit high levels of off-target toxicity that limit their employment for certain groups of patients. Thus, there remains an unmet medical need that could potentially be resolved with the development and utilization of neutralizing human anti-HCMV antibodies derived from immune individuals.

This project aims to explore the human antibody repertoire against HCMV and to develop fully human anti-HCMV antibodies. A human immune antibody phage display library consisting of about 18.5 million unique Fab clones was constructed from a blood donor with a high titer of anti-HCMV antibodies. This library has been panned against whole viral particles of AD169 and RV1305 strains. The panning process led to the discovery of 31 unique HCMV-specific Fab-expressing phages which were then converted to full-length human IgG1 antibodies. Results show that some of their targets include the major capsid protein (UL86) and pp65. Furthermore, flow cytometry results reveal that one of these antibodies, 3A11, target a HCMV antigen displayed on the surface of infected MRC-5 and ARPE-19 cells during the lytic phase of HCMV infection. Analysis by western blot suggests that this antigen is expressed as early as 12h post infection.

Future work encompasses the detailed delineation of epitopes targeted by 3A11 and the examination of its potential to mediate ADCC and complement activation. This work has important implications for our understanding of natural human immunity against HCMV and can potentially impact upon the design of future viral vaccines.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION
CD-PP-011

Characterization of epitope-specific humoral responses to human cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in solid organ transplant recipients

Ilona Anna Baraniak¹, Barbara Kropff², Gary R McLean³, Richard Milne¹, Sylvie Pichon⁴, Matthew Reeves¹, Michael Mach², Paul Griffiths¹

¹University College London, Institute of Immunity and Transplantation, LONDON, United Kingdom

²Institut für Klinische und Molekulare Virologie Friedrich-Alexander-Universität, ERLANGEN, Germany

³Cellular and Molecular Immunology Research Centre, London Metropolitan University, LONDON, United Kingdom

⁴Clinical Development, Sanofi Pasteur, MARCY L'ETOILE, France

The human cytomegalovirus (HCMV) virion envelope protein glycoprotein B (gB) is essential for viral entry and represents a major target for humoral responses following infection. To assess whether vaccination with gB plus MF59 adjuvant could be protective, a phase-2 clinical trial (NCT00299260) was conducted in solid organ transplant candidates. Vaccination of both seronegative and seropositive recipients significantly increased gB antibody levels measured by ELISA whose titre correlated directly with protection against post-transplant viraemia. The aim of this current study was to investigate the precise nature of the protective humoral response in vaccinated transplant recipients. We focussed on four key antigenic domains (AD) of gB; AD1, AD2, AD4 and AD5 measuring the antibody response against these regions in patient sera and correlating this with viraemia. Vaccination of seropositive patients significantly boosted antibody responses against the immunodominant region AD1 as well as towards AD2, AD4 and AD5. Approximately 50% of patients had detectable responses to AD2 at baseline and while vaccination enhanced these pre-existing responses it failed to induce de novo AD2 responses in seropositives. Interestingly, the boost to AD2 correlated with a decreased incidence of viraemia in these patients consistent with antibodies against this epitope being protective. Analysis of sera from seronegative vaccinees revealed that de novo antibody responses were restricted to AD1 and AD5 with neither correlating with protection. Overall, these data support responses to AD2 contributing to immune protection of seropositives but not seronegatives following vaccination with gB.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-026

Influence of HCMV pentameric complex-specific depletion on neutralizing capacity of immunoglobulin preparations

Matthias Stefan Schampera, Jose Arellano-Galindo, Gerhard Jahn, Klaus Hamprecht
University Tuebingen, Institute of Medical Virology and Epidemiology of Viral Diseases, TUEBINGEN, Germany

Introduction: In the absence of a highly-efficient HCMV-vaccine and in context of the recent RCT-study of Revello et al., 2014, which could not confirm the preventive effects of HCMV-specific hyperimmunoglobulins (HIG) on maternofetal HCMV-transmission as reported originally by Nigro et al., 2005, we analyzed the neutralization-capacity (NC) of HIGs and standard-IVIGs in vitro by depletion of pentameric complex (PC) related antibodies.

Objectives: We investigated the NC-changes using a recombinant-HCMV-PC and defined UL130 peptides in order to analyze their contribution to NC.

Methods: We analyzed the NC in vitro using the HIG-preparation Cytotect® and a standard-intravenous-immunoglobulin (IVIG) Kiovig®. The depletion of antibodies was performed using a recombinant-PC and two UL130-peptides. We used a six-fold histidine-tail on the PC, which was linked to magnetic-beads containing His-Tag-mouse-mAbs on the surface. We used a protocol for a long-term cell-free HCMV-neutralization-plaque-reduction-assay (PRA) using human foreskin fibroblasts (HFF) and human retinal epithelial cells (ARPE-19) cells as target cells. Each primary HFF- and ARPE-19-adapted clinical-isolate from amniotic-fluid was propagated until cell-free HCMV-shedding, respectively. For calibration we previously generated serum-pools (N=100) from two cohorts of mothers: seronegative and latently HCMV infected mothers at birth. For control, untreated immunoglobulin preparations were investigated.

Results: HIG and standard IVIG-preparations lead to a significant decrease in NC after incubation with the recombinant PC compared to untreated samples. However, the impact of depletion was not significant against infected HFF-monolayers.

Conclusion: We demonstrated that polyclonal HCMV-specific antibodies present in HIG and standard IVIGs bind to the recombinant PC and therefore may play an important role for the neutralization-activity of HIG and IVIG-preparations. The antibodies targeting the recombinant PC are more effective for viral neutralization in ARPE-19- than in HFF-microcultures. Further epitope analyses of the PC-binding NT-antibodies could lead to a better understanding of specificity and efficiency of these antibodies to prevent potentially maternofetal HCMV-transmission.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-027

Glycoprotein B subunit immunization elicits limited IgG responses against defined gB neutralizing epitopes

Cody Nelson¹, Eduardo Cisneros de la Rosa¹, Meng Chen¹, Nathan Vandergriff¹, Robert Pass², Sallie Permar¹

¹Duke University Medical Center, Human Vaccine Institute, DURHAM, United States of America

²University of Alabama Birmingham, Department of Pediatrics, BIRMINGHAM, United States of America

A vaccine to prevent maternal acquisition of human cytomegalovirus (HCMV) during pregnancy is one potential strategy to reduce the incidence of infant congenital CMV disease. The glycoprotein B (gB) subunit + MF59 adjuvant vaccine platform has demonstrated a promising 50% efficacy in preventing

primary HCMV acquisition in multiple phase II clinical trials, yet the mechanism of vaccine protection is unknown. 33 unique patient plasma samples from the postpartum gB/MF59 vaccine trial cohort (each at or near peak immunogenicity) were assessed for their vaccine-elicited antibody binding and neutralizing function, and these data (vaccinee=V) were compared to that of a chronically-infected (seropositive=SP) control cohort. gB/MF59 vaccination elicited high-magnitude gB-specific antibodies (median V $\log_{10}ED_{50}=3.96$) with a high degree of affinity maturation (median V relative affinity index [RAI]=0.91), comparable to the gB-specific antibody responses elicited by natural infection (median SP $\log_{10}ED_{50}=3.30$; RAI=0.96). Additionally, the IgG subclass distribution was similar between vaccinees and seropositive controls, with robust IgG₁ and IgG₃ responses noted in each group but no detectable IgG₂ or IgG₄ responses. However, in contrast to that of chronic infection, gB/MF59-elicited antibodies exhibited negligible neutralizing function (epithelial cell median ID₅₀: V<30; SP=603; *p<0.001, Wilcoxon test) and rare binding antibody responses against multiple gB protein structural motifs recently-described as the target of neutralizing antibodies, including AD-2 (% responders: V=9%; SP=40%; *p=0.023, Fisher's Exact test) and Domain I (% responders: V=36%; SP=83%; *p=0.002, Fisher's Exact test). Of note, there was no clear difference of gB epitope binding responses between vaccinees who became infected and those who did not. Altogether, this data suggests that antibody responses against gB neutralizing epitopes may not have been responsible for the partial efficacy of gB/MF59 vaccination, and that non-neutralizing functional antibody responses, such as antibody-dependent cellular cytotoxicity, could have played a role in the observed 50% protection.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-038

Pharmakokinetics of hyperimmune globulin (HIG) administration for prevention of maternofetal CMV-transmission in primary infected pregnant women

Klaus Hamprecht, Imma Fischer, Christoph Meisner, Gerhard Jahn, Karl Oliver Kagan
University Hospital of Tuebingen, Institute of Medical Virology, TUEBINGEN, Germany

Introduction: In the absence of an efficient vaccine, the administration of HIG is an option beside the education of pregnant women on prevention strategies of CMV transmission. The important contributions of Revello et al 2014 (RCT on HIG treatment) and 2015 (observational controlled study on hygiene information) have to be acknowledged.

Methods: Volunteers of CMV primary infected pregnant women up to a gestational age of 20 weeks were included in our observational study. At study entry, CMV serology, epithelial cell-specific neutralisation plaque-reduction assays (PRNT) were performed. At defined time points (directly before, 30 min after, 2h after, 7 days after, 14 days after) we analysed CMV-IgG, IgG-avidity, and PRNT. In a subcohort of 5 pregnant mothers a still more comprehensive kinetic analysis was performed.

Results: Based on anti-HBs data of Biotest BT 507 of Thürmann et al., a terminal CMV elimination half-life of 22,1 days was assumed in 1995. Therefore, all relevant HIG-studies used a treatment-interval of 4 weeks. However, we have shown for an index-case, that these original data are not consistent using new CMV-IgG test systems for analysis of CytotectO (Hamprecht et al., NEJM 2014). We compared the CMV-IgG kinetics of two groups of mothers with different GAs using a two-compartment model of HIG-elimination. For 18 groupI-mothers (GA<16weeks) we found an initial half-life (ihl) of 12,7 days, while the terminal half-life (thl) was 20,1 days, compared to 23 groupII-mothers with ihl of 13,5 versus thl 18,8 days. Evaluating the anti-HbS IgG data of unvaccinated individuals of both study-groups showed the proof of principal.

Conclusions: Our study demonstrates unequivocally that the initial data of Thürmann et al. were misleading. Therefore, using our modified study design, we can demonstrate impressively, that HIG may be a very potent prevention method for maternofetal CMV-transmission in the first trimenon (see Kagan et al.).

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-039

Effectiveness of a 2 weekly HIG protocol in first trimester CMV infection

Karl Oliver Kagan, Klaus Hamprecht, Markus Hoopmann, Rangmar Goelz, Gerhard Jahn
University Hospital of Tuebingen, Obstetrics and Gynaecology, TUEBINGEN, Germany

Introduction:

Primary CMV infection is particularly associated with an adverse outcome if transmission occurs before 20 weeks. Administration of HIG should prevent transmission but the results are contradictory. So far, HIGs are given on a 4weekly basis. However, we were able to demonstrate an index case with a half-life of about 14days (Hamprecht et al. 2014) In this pilot-study we examine the effectiveness of a HIG on a 2weekly basis in primary CMV infection in the first trimester.

Methods:

This is an observational study at the University Hospital of Tuebingen between 2013 and 2016. Women with a primary infection \leq 14 weeks were included. A recent infection was assumed if $<40\text{U/ml}$, IgM-Index >2 , CMV IgG avidity $<40\%$, and without presence of anti-CMV-gB2-IgG. HIG application was started as soon as possible and repeated every 2 weeks. HIG was stopped when CMV-IgG levels were stable $> 100\text{U/ml}$ after 2 weeks of the last HIG cycle (Hamprecht et al., 2017).

In all cases, an amniocentesis was performed at around 20 weeks.

Amniotic fluid was tested by nested PCR, q-rt-PCR and 18h and 14d cell culture. Results are given as mean.

Results:

20 women fulfilled the inclusion criteria. Serostatus was examined at 11.6weeks' gestation and HIG application was started at 13.6weeks, respectively. In 20cases HIG were administered twice, in 18, 9, 3 and 1 case, HIG was given 3, 4 and 5 times, respectively. Mean gestational age at administration was 15.2, 17.0, 17.8 and 18.4weeks. Amniocentesis was performed at 20.1weeks. In none of the cases, we observed transmission.

Conclusion:

Administration of HIG may play a role in the prevention of first and second trimester transmission if they are given on a 2 weekly basis. Further, larger studies are necessary to confirm this finding.

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Date: 03/05/2017

Time: 16:00 - 17:00 hrs

POSTER PRESENTATION

CD-PP-042

The presence of cytomegalovirus-DNA in the uterine cervical secretion is an independent predictor of congenital infection in high-risk pregnant women

Kenji Tanimura¹, Shinya Tairaku¹, Yasuhiko Ebina¹, Ichiro Morioka¹, Satoshi Nagamata¹, Kana Ozaki¹, Mayumi Morizane¹, Masashi Deguchi¹, Toshio Minematsu², Hideto Yamada¹

¹Kobe University Graduate School of Medicine, Department of Obstetrics and Gynecology, KOBE, Japan

²Aisenkai Nichinan Hospital, Research Center for Disease Control, MIYAZAKI, Japan

Objectives:To determine maternal clinical, laboratory, and ultrasound (US) findings that effectively predict the occurrence of congenital cytomegalovirus (CMV) infection (CCI) in high-risk pregnant women.

Methods: Three hundred CMV IgM-positive pregnant women were enrolled in a prospective cohort study. The maternal clinical and laboratory findings, including serum CMV IgM and IgG, IgG avidity index (AI), C7-HRP testing, and polymerase chain reaction (PCR) for the detection of CMV-DNA in the maternal serum, urine, and uterine cervical secretion, and prenatal US findings were evaluated. To

determine predictive factors for the occurrence of CCI, univariate and multivariable logistic regression analyses were performed.

Results: In 22 of the 300 women, CCI was confirmed using PCR for CMV-DNA in newborn urine. Univariate analyses demonstrated that the presence of maternal flu-like symptoms, presence of US fetal abnormalities, serum titers of CMV IgM, positive results for C7-HRP, CMV IgG AI <40%, and positive PCR results in the uterine cervical secretion were statistically associated with the occurrence of CCI. Multivariable analysis revealed that the presence of US fetal abnormalities (OR 31.9, 95% CI 8.5–120.3; $p < 0.001$) and positive PCR results in the uterine cervical secretion (OR 16.4, 95% CI 5.0–54.1; $p < 0.001$) were independent predictive factors of CCI in CMV IgM-positive women.

Conclusions: This prospective study demonstrates for the first time that the presence of CMV-DNA in the maternal uterine cervical secretion and US fetal abnormalities was predictive of the occurrence of CCI in high-risk pregnant women.

470 - Session B.06: Virus and Host

Date: 03/05/2017

Time: 17:00 - 18:15 hrs

B-INV-006

Antibody and/or T-cell driven design for a protective HCMV vaccine?

Daniele Lilleri¹, Maria Grazia Revello¹, Federico Mele², Laurent Perez², Federica Sallusto², Antonio Lanzavecchia², Giuseppe Gerna¹

¹Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy

²Institute for Research in Biomedicine, Università della Svizzera italiana, BELLINZONA, Switzerland

A breakthrough in the development of HCMV vaccine was represented by the identification of the pentamer complex (PC) gHgLPUL128L, which is required for infection of epithelial/endothelial cells and virus transfer to leukocytes. The analysis of the neutralizing antibody response to HCMV identified the PC as the most suitable vaccine candidate. Serum neutralizing activity against HCMV is mainly directed against the PC. Antibody directed to the PC are also able to block *in vitro* cell-to-cell HCMV spreading and syncytium formation in epithelial cells, and transfer of HCMV from infected endothelial cells to leukocytes. The different approaches adopted to develop a PC including vaccine comprise a non-replicating AD169 vaccine with the PC restored, modified vaccinia Ankara or alpha virus replicon expressing the PC, or recombinant PC produced by a stably transfected CHO cell line. Results of animal immunization showed that PC elicited significantly higher levels of neutralizing antibodies than gH/gL or gB. The subunit PC elicited in mice HCMV neutralizing antibody titers 100-1000 fold higher than those found in individuals recovering from HCMV infection. These results indicate that the PC is a strong candidate for a vaccine capable of selectively inducing a broad and potent protective antibody response. However, it should be reminded that an efficient T cell response is mandatory for control of HCMV infection in transplant recipients and neutralizing antibodies alone are not protective *in vivo*. A rapid development of HCMV-specific T cell response is also associated with a lower risk of transmission of HCMV to the fetus after primary infection in pregnancy. Recently we started to investigate the T-cell response elicited by PC after HCMV infection, in order to verify whether a PC-based vaccine could also be able to elicit an effective T cell response, or other viral antigens are required.

470 - Session B.06: Virus and Host

Date: 03/05/2017

Time: 17:00 - 18:15 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-021

The endothelin B receptor is critical for human cytomegalovirus infection in endothelial cells and a potential target for anti-viral therapy

Masany Jung¹, Koon-Chu Yaiw¹, Tim Schulte¹, Olov Wahlsten², Abdul-Aleem Mohammad¹, Alice Assinger³, Lisa Simonsson Nyström², Helena Costa¹, Huanhuan Leah Cui¹, Vanessa Wilhelmi¹, Hudson

Pace², Belghis Davoudi¹, Giorgos Tsipras¹, Patrick Scicluna¹, Natalia Landazuri¹, Ourania Kostopoulou¹, Sharan Ananthaseshan¹, Suhas Vasaikar¹, Chato Taher¹, Lynn Butler¹, Afsar Rahbar¹, Hjalmar Brismar⁴, Andrea Carfi⁵, Tomoko Doi⁶, Jiri Bartek¹, Kum-Thong Wong⁷, Per-Åke Nygren⁴, Fredrik Höök², Adnane Achour¹, Cecilia Söderberg-Nauclér¹

¹Karolinska Institute, STOCKHOLM, Sweden

²Chalmers University of Technology, Department of Applied Physics, GOTHENBURG, Sweden

³Medical University of Vienna, Institute of Physiology, VIENNA, Austria

⁴KTH Royal Institute of Technology, Department of Applied Physics, STOCKHOLM, Sweden

⁵GSK Vaccines, ROCKVILLE, United States of America

⁶Kyoto University, Department of Biophysics, KYOTO, Japan

⁷University of Malaya, Department of Pathology, KUALA LUMPUR, Malaysia

Human cytomegalovirus (HCMV) is the major infectious agent responsible for congenital malformations, is associated with severe or fatal disease in immunocompromised patients, and is linked to atherosclerosis and certain malignancies. Cellular receptors that mediate entry of HCMV into fibroblasts and neuronal cells have been identified, but the main receptor for entry into the major cell types infected in vivo—endothelial, epithelial, and myeloid lineage cells—is unknown. Entry into those cell types is believed to be mediated via the pentameric complex consisting of gH, gL, UL-128, UL130 and UL131. In this study, we found that the endothelin B receptor (ETBR) is critical for HCMV infection in endothelial, epithelial, and myeloid lineage cells through a mechanism involving signaling by G-protein-coupled receptors (GPCRs) and interaction with the viral pentameric complex. In endothelial cells, silencing of ETBR with siRNA significantly prevented HCMV infection, and ETBR-specific antagonists reduced HCMV infection by 80-99%. In fibroblasts, ETBR antagonists had little or no inhibitory effect on infection. However, in endothelial cells, pretreatment with ETBR antibody or ETBR protein significantly reduced HCMV infection. Transfection of ETBR into human or mouse cells increased production of HCMV transcripts and interestingly also viral particles. Total internal reflection fluorescence microscopy confirmed a specific interaction between HCMV and ETBR that was reduced by the ETBR ligand endothelin-1, anti-ETBR antibody, or ETBR antagonist. Microscale thermophoresis and co-immunoprecipitation assays corroborated the physical binding of the viral pentameric complex and ETBR. Our findings show that ETBR is important in the HCMV life cycle and a potential target for antiviral therapy.

470 - Session B.06: Virus and Host

Date: 03/05/2017

Time: 17:00 - 18:15 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-022

Late-rising CD4 T cells resolve CMV persistence

Chris A Benedict¹, Bryan McDonald¹, Gregory Seumois¹, Rachid El Morabiti¹, Raima Ghosh¹, Zheng Fu¹, Barbara Adler², Pandurangan Vijayanand¹, Ankan Gupta¹

¹La Jolla Institute for Allergy and Immunology, LA JOLLA, United States of America

²Max Von Petternkoffer-Institute for Virology, MUNICH, Germany

Robust CD4 T cell responses help to control chronic infection and restrict disease, but their specific requirements for resolving viral persistence remain poorly defined. During mouse cytomegalovirus (MCMV) infection, CD4 T cells recognizing an epitope derived from the viral M09 protein expand at late times of infection and display a unique phenotype compared to their conventional counterparts, including high IFN γ -production and low expression of the activated CD43 isoform. Ablating these late-rising CD4 T cells by mutating the MCMV M09 genomic epitope, or inducing them through vaccination, revealed their critical role in resolving persistent replication by overcoming IL-10 mediated immune suppression. Together, these data show that unique subsets of late-rising CD4 T cells are qualitatively superior in resolving chronic infection when compared to those that expand earlier, results that should be considered for vaccine development.

470 - Session B.06: Virus and Host

Date: 03/05/2017

Time: 17:00 - 18:15 hrs

BOTH ORAL & POSTER PRESENTATION

B-OP-023

In Wildtype HCMV, the Pentameric Complex Drives Efficient Direct Cell-Cell Transmission that is Resistant to Host Immune Defenses

Richard Stanton, Isa Murrell, Carmen Bedford, Kristin Ladell, Kelly Miners, David Price, Peter Tomasec, Gavin Wilkinson, [Richard Stanton](#)
Cardiff University, Infection & Immunity, CARDIFF, United Kingdom

Human cytomegalovirus (HCMV) strains passaged *in vitro* rapidly acquire mutations that impact viral growth. These laboratory-adapted strains of HCMV generally exhibit restricted tropism, produce high levels of cell-free virus and develop susceptibility to natural killer (NK) cells. To permit experimentation with a genetically intact virus, that retained the phenotype of clinical virus, a wild-type (wt) strain Merlin genome was reconstructed using bacterial artificial chromosome (BAC) technology. Like clinical virus, this genome proved to be unstable in cell culture, however propagation of intact virus was achieved by placing the RL13 and UL128 genes under conditional expression. Here we show that wt-HCMV produces extremely low titres of cell-free virus, similarly to clinical virus. However a novel cell-cell transfer assay demonstrated that this virus efficiently infected fibroblasts, epithelial, monocyte-derived dendritic and Langerhans cells via direct transmission. This process of cell-cell transfer required the UL128 locus, but not the RL13 gene, and was significantly less vulnerable to the disruptive effects of interferon, restriction factors, and neutralizing antibodies, in multiple cell types, compared with cell-free entry. Resistance to neutralising antibodies was dependent on high level expression of a pentameric gH/gL/gpUL128-131A complex, a feature unique to wt but not passaged strains of HCMV. Cell-cell transfer is likely to be the dominant route of intra-host spread, thus the demonstration that wt-HCMV is uniquely resistant to aspects of innate, adaptive and intrinsic immune defences, underlines the importance of testing interventions against genetically intact strains.

510 - Session D.01: Treatment & Prevention

Date: 04/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

D-OP-001

Live-CMV vaccines lacking viral MHC I homologs are attenuated in guinea pigs but confer sterilizing immunity against congenital CMV transmission

[Mark Schleiss](#)¹, Pete Gillis¹, Craig Bierle¹, Kaitlyn Anderholm¹, Nelmary Hernandez-Alvarado¹, Jian Ben Wang², Zainab Al-Mahdi², Michael McVoy²

¹Centre for Infectious Diseases and Microbiology Translational Research, University of Minnesota Medical School, Department of Pediatrics, MINNEAPOLIS, United States of America

²Virginia Commonwealth University Medical School, Department of Pediatrics, RICHMOND, VIRGINIA, United States of America

Cytomegaloviruses evade NK cells by expressing viral major histocompatibility class I (MHC I) homologs. Guinea pig cytomegalovirus (GPCMV) encodes three putative MHC I homologs: gp147, gp148, and gp149. A GPCMV lacking all three MHC I homologs was attenuated *in vivo* but retained immunogenicity and efficacy comparable to parental wild type (WT) virus when used as a live vaccine (Crumpler, *Vaccine* 27, 2009). In the present study, mRNAs encoding gp147, gp148, or gp149 were detected during viral infection and gp147 and gp149 were glycosylated when transiently expressed as epitope-tagged proteins. Viruses with individual gene deletions designated Δ 147, Δ 148, and Δ 149 were evaluated *in vivo*. Δ 148 had no discernable phenotype. Δ 147 and Δ 149 were cleared from blood 7 days earlier than WT and failed to induce transient elevation of blood IFN γ mRNA that occurred following WT infection ($p < 0.01$). Neutralizing and GPCMV-binding antibodies induced by Δ 147 and Δ 149 were lower on day 14 but indistinguishable from WT on days 28 and 70 post-inoculation. In a pre-conception

vaccination/mid-gestation challenge model, efficacy of vaccination with $\Delta 147$ or $\Delta 149$ was comparable to WT. Maternal viremia was reduced from 9.3×10^5 copies/ml in naïve controls to <100 copies/ml in vaccinated animals, and fetal infection declined from 35% in naïve animals to 0% in all three vaccine groups ($p < 0.01$). Average pup weight improved from 65 grams for pups born to naïve dams to 89, 92, or 90 grams for pups born to $\Delta 147$ -, $\Delta 149$ -, or WT-immunized dams, respectively ($p < 0.001$). Pup mortality declined from 83% in naïve dams to 0, 10, and 21% in $\Delta 147$ -, $\Delta 149$ -, or WT-vaccinated dams, respectively ($p < 0.0001$). These results are consistent with the hypothesis that gp147 and gp149 function as NK evasins and suggest that deletion of analogous MHC I homologs could enhance the safety and immunogenicity of a live human cytomegalovirus vaccine.

510 - Session D.01: Treatment & Prevention

Date: 04/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

D-OP-002

Active evolution of memory B-cells specific for viral gH/gL/pUL128-131 pentameric complex in healthy subjects with silent human cytomegalovirus infection

Tong-Ming Fu

Merck Sharp and Dohme Company, WEST POINT, United States of America

Human cytomegalovirus (HCMV) is an important pathogen as it can cause life-threatening infection in immunosuppressed patients, but also *in utero* infection that may lead to birth-defects. No prophylactic vaccine is available. HCMV infection in healthy subjects is generally asymptomatic and virus establishes latent infection that persists for life. Host immunity to HCMV is effective against reactivation infection and super-infection with a new strain. Thus, vaccine candidates able to elicit immune responses similar to those of natural infection may confer protection. Since neutralizing antibodies are essential for prophylactic vaccines, it is important to understand how antiviral antibodies are developed in the context of natural infection. We hypothesize that developmental path of neutralizing antibodies in seropositive subjects can be deciphered by interrogating respective host B-cell repertoires using unique genetic signature sequences of monoclonal antibodies (mAbs). Towards this goal, we isolated 56 HCMV-specific mAbs from three healthy donors with high, medium and low levels of serum neutralizing titers. The mAbs specific for the pentameric complex of gH/gL/pUL128-131 were more potent in viral neutralization as opposed to those of gB. Using these mAbs as probes, patterns of extended lineage development for B-cells, genetic evidence of active antibody maturation, were revealed in two donors with high and medium titers. Importantly, the patterns of extended lineage evolution were limited to five mAbs specific to the pentamer, none of gB mAbs. Thus, memory B-cells of antiviral function such as neutralization were active during latency in healthy donors, potentially correlated with donors' higher neutralizing titers. Also, our results indicated that memory B-cells of neutralization could be frequently mobilized, probably responding to silent asymptomatic viral episodes in the host, and further suggesting that neutralizing antibodies could play a role in control of recurrent infection.

510 - Session D.01: Treatment & Prevention

Date: 04/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

D-OP-003

The breadth of synthetic long peptide vaccine-induced CD8+ T cell responses determines the efficacy against mouse cytomegalovirus infection

Eleni Panagioti¹, Anke Redeker², Suzanne Van Duikeren², LMC Kees Franken², Jan Wouter Drijfhout², Sjoerd Van der Burg², Ramon Arens²

¹LUMC, Medical Oncology, LEIDEN, Netherlands

²Leiden University Medical Center, LEIDEN, Netherlands

There is an ultimate need for efficacious vaccines against human cytomegalovirus (HCMV), which causes severe morbidity and mortality among neonates and immunocompromised individuals. In this study we explored synthetic long peptide (SLP) vaccination as a platform modality to protect against CMV infection in preclinical mouse models. In both C57BL/6 and BALB/c mouse strains, prime-booster vaccination with SLPs containing MHC class I restricted epitopes of mouse CMV (MCMV) resulted in the induction of strong and polyfunctional (i.e., IFN- γ^+ , TNF $^+$, IL-2 $^+$) CD8 $^+$ T cell responses, equivalent in magnitude to those induced by the virus itself. SLP vaccination initially led to the formation of effector CD8 $^+$ T cells (KLRG1 hi , CD44 hi , CD127 lo , CD62L lo), which eventually converted to a mixed central and effector-memory T cell phenotype. Markedly, the magnitude of the SLP vaccine-induced CD8 $^+$ T cell response was unrelated to the T cell functional avidity but correlated to the naive CD8 $^+$ T cell precursor frequency of each epitope. Vaccination with single SLPs displayed various levels of long-term protection against acute MCMV infection, but superior protection occurred after vaccination with a combination of SLPs. This finding underlines the importance of the breadth of the vaccine-induced CD8 $^+$ T cell response. Thus, SLP-based vaccines could be a potential strategy to prevent CMV-associated disease.

510 - Session D.01: Treatment & Prevention

Date: 04/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

D-OP-004

Novel antivirals for the prevention and treatment of congenital CMV

Stuart Hamilton¹, Corina Hutterer², Jens Milbradt², Ece Egilmezer³, Manfred Marschall², William Rawlinson³

¹Virology Research Laboratory, SEALS Microbiology, SYDNEY, Australia

²Institute for Clinical and Molecular Virology, Friedrich-Alexander University, ERLANGEN-NURNBERG, Germany

³Virology Research Laboratory, SEALS Microbiology, University of New South Wales, SYDNEY, Australia

Introduction: CMV causes congenital malformation and fetal death. There are no licensed therapeutics for prevention or treatment of CMV infection during pregnancy due to toxicity and limited efficacy. Promising experimental anti-CMV compounds (maribavir, letermovir, brincidofovir) and novel compounds (quinazoline derivative-vi7392, novel cellular kinase inhibitor-Sc88941 and novel dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) inhibitors) were investigated in our ex vivo placental models. **Methods:** The efficacy of current experimental and novel antivirals were studied at therapeutic concentrations (2.5 μ M) in vitro in Merlin-infected (1 pfu/cell) TEV-1 trophoblasts cells and AD169- and Merlin-infected explanted human multicellular placental models. Plaque assays, qPCR, immunofluorescence and western blot analyses were performed. **Results:** No cellular toxicity associated with antiviral treatment was observed in TEV-1 or placental cultures at the concentration used. Treatment of TEV-1 cells with maribavir, letermovir, cidofovir, vi7392, Sc88941, DYRK inhibitors or the reference ganciclovir significantly inhibited virus progeny production 7 days post infection (dpi) relative to untreated cells (89%, 82%, 74%, 69%, 99%, 97% and 64% inhibition respectively, $p < 0.05$). Treatment of CMV-infected ex vivo placental explants at 5dpi with maribavir, letermovir, cidofovir, vi7392, Sc88941, DYRK inhibitors or ganciclovir also significantly inhibited CMV replication relative to untreated placental explants at 19dpi ($p < 0.05$). Immunofluorescence and western blot analyses showed antiviral treatment inhibited CMV protein expression and viral dissemination. CMV infection induced focal upregulation of DYRK1A and DYRK1B predominately within the cytoplasm and nucleus respectively in CMV-infected cell cultures, placental explants and clinical placental tissue. **Conclusion:** Current experimental and novel anti-CMV compounds may be a viable therapeutic for use during pregnancy. The novel compounds need to be further studied for fetal toxicity in animal models. Even with effective vaccination, antivirals will continue to be needed for infections of pregnancy and immunosuppressed patients for at least 30 years.

510 - Session D.01: Treatment & Prevention

Date: 04/05/2017

Time: 08:30 - 10:25 hrs

BOTH ORAL & POSTER PRESENTATION

D-OP-005

A CMV vaccine based on non-replicating lymphocytic choriomeningitis virus vectors expressing gB and pp65 is safe and immunogenic in man

Anders Lilja¹, Michael Schwendinger¹, Georges Thiry¹, Beatrice De Vos², Fien De Bouver³, Geert Leroux-Roels³

¹Hookipa Biotech AG, VIENNA, Austria

²BEJAMAD sprl, DWORP, Belgium

³Ghent University Hospital, CEVAC, GHENT, Belgium

HB-101 is a CMV vaccine candidate based on non-replicating lymphocytic choriomeningitis virus vectors expressing two human CMV antigens, the tegument protein pp65 and a truncated isoform of the fusion protein gB. The safety and immunogenicity of HB-101 were evaluated in a randomized, placebo-controlled, double-blind phase I dose-escalating trial (NCT02798692). Three cohorts of 18 subjects per cohort were enrolled. In each cohort, 14 subjects received the vaccine and four received placebo. Vaccine and placebo were administered on day 0, month 1, and month 3. Safety and reactogenicity data were collected and reviewed by an independent data and safety monitoring board. Immunogenicity readouts included humoral and cellular responses against gB, cellular responses against pp65, as well as humoral and cellular responses against the LCMV vector backbone. Overall, HB-101 was safe and elicited potent immune responses. Unblinded safety and immunogenicity data through month 4 of the study will be presented and will be the first public disclosure of these results.

530 - Session D.02: Treatment & Prevention

Date: 04/05/2017

Time: 10:55 - 12:20 hrs

BOTH ORAL & POSTER PRESENTATION

D-OP-006

CMV-specific CD4+ T cells in CMV-IgG-seronegative individuals protect from CMV viremia following transplantation with a CMV-seropositive donor kidney

Nicolle Litjens, Ling Huang, Burc Dedeoglu, Ruud Meijers, Michiel Betjes

Erasmus Medical Center, Internal Medicine, Nephrology and Transplantation, ROTTERDAM, Netherlands

A primary infection with cytomegalovirus (CMV) is one of the major threats following transplantation of a CMV-IgG-seropositive donor organ into a CMV-IgG-seronegative individual. Therefore, prophylactic treatment with valganciclovir is given in these individuals. However, CMV-specific T-cell immunity may exist without measurable anti-CMV IgG. The frequency and clinical relevance of solitary CMV-specific T-cell immunity is not known. The aim of this study is to assess the frequency of solitary CMV-specific T cells in a cohort of CMV-IgG-seronegative individuals and the clinical relevance with respect to CMV-infection following transplantation.

In a cohort of 28 CMV-IgG-seronegative and 14 CMV-IgG-seropositive individuals, CMV-specific cytokine-producing and proliferating T cells were assessed prior to transplantation using the CD137 multi-parameter assay and CFSE-dilution, respectively. CMV-specific humoral immunity was evaluated using the B-cell ELISPOT assay.

In 46% of CMV IgG-seronegative individuals CMV-specific CD137⁺IFN- γ -producing CD4⁺ T cells were detected above background (median values amounted to 0.01% versus 0.58% in CMV-IgG-seropositive individuals). CMV-specific proliferating CD4⁺ T cells were detected above background in 55% of the CMV-IgG-seronegative individuals (median values amounted to 0.4% versus 6.34% in CMV-IgG-seropositive individuals). CMV-specific IgG-producing antibody secreting cells (ASC) were barely detected in CMV-IgG-seronegative individuals (median values amounted to 3/10⁵ cells versus 48/10⁵

cells in CMV-IgG-seropositive individuals). However, a positive association was observed for CMV-specific CD137⁺IFN- γ -producing CD4⁺ T cells and CMV-specific IgG ASC (Rs=0.52, P<0.05). In 46% of CMV IgG-seronegative individuals a CMV-viremia developed following transplantation. CMV-specific CD137⁺IFN- γ -producing CD4⁺ T cells were associated with protection from a CMV-viremia following transplantation, i.e. positive responses were detected in 10/15 non-viremic versus 3/13 viremic recipients of a kidney transplant from a CMV-IgG-seropositive donor (P=0.02).

A solitary CMV-specific T-cell response without detectable anti-CMV antibodies is frequent and clinically relevant as it yields significant protection to infection following transplantation with a kidney from a CMV-IgG-seropositive donor.

530 - Session D.02: Treatment & Prevention

Date: 04/05/2017

Time: 10:55 - 12:20 hrs

BOTH ORAL & POSTER PRESENTATION

D-OP-007

KLRG1+ CD8 T cells induced by cytomegalovirus vector expressing NKG2D ligand RAE-1? provide robust protection against tumor challenge

Tihana Trsan¹, Kristina Vukovic¹, Niels A. Lemmermann², Kilian Schober³, Dirk H. Busch³, Martin Messerle⁴, Astrid Krmpotic¹, Stipan Jonjic¹

¹Faculty of Medicine, University of Rijeka, Department of Histology and Embryology / Center for Proteomics, RIJEKA, Croatia

²University Medical Center of the Johannes Gutenberg-University Mainz, Institute for Virology, MAINZ, Germany

³Technische Universität München (TUM), MUNICH, Germany

⁴Hannover Medical School, Department of Virology, HANNOVER, Germany

Although T cell therapy has been proven as a successful approach against certain tumors, an efficient CD8 T cell tumor vaccine is still not available. Here we demonstrate that murine cytomegalovirus (MCMV) expressing NKG2D ligand RAE-1 γ (RAE-1 γ MCMV) possesses an exceptional capacity to serve as CD8 T cell-based tumor vaccine. Immunization with the RAE-1 γ MCMV vector expressing SIINFEKL epitope postponed, or completely prevented the growth of B16 melanoma cells expressing ovalbumin. The protection against melanoma challenge was demonstrated in both, prophylactic and therapeutic approaches. Expression of RAE-1 γ by the MCMV vector potentiated induction of KLRG1-expressing SIINFEKL-specific CD8 T cells with enhanced effector properties, ensuring long-term protection, even against a secondary tumor challenge. This approach allowed further improvement of CD8 T cell properties by checkpoint therapy, resulting in a postponed tumor development and increased survival. Moreover, vaccination with the RAE-1 γ MCMV vector successfully induced protective CD8 T cells even when applied in immunologically immature newborn mice, providing protection against tumor challenge throughout their adult life. Altogether, our data demonstrate that RAE-1 γ -expressing CMV vector represents a powerful base for the development of CD8 T cell tumor vaccines.

530 - Session D.02: Treatment & Prevention

Date: 04/05/2017

Time: 10:55 - 12:20 hrs

BOTH ORAL & POSTER PRESENTATION

D-OP-008

Disabled Infectious Single Cycle (DISC) Cytomegalovirus Vaccine Encoding Pentameric Complex is Completely Protective Against Congenital Guinea Pig CMV

Alistair McGregor, K. Yeon Choi

Texas A&M University Health Science Center, Microbial Pathogenesis & Immunology, COLLEGE STATION, United States of America

The guinea pig is the only small animal model for congenital CMV (cCMV). We investigated a novel disabled infectious single cycle (DISC) vaccine strategy, where the virus lacks the ability to express an essential capsid gene (GP85), except when grown on a complementing cell line (GPLtet-off). Using this strategy, two DISC GPCMV viruses were generated: 1) GP85DISC, lacking pentameric complex (PC), 2) GP85DISCPC+, with PC. Vaccine strains were evaluated for immunogenicity and tested for efficacy against wild type virus cCMV. Both vaccines induced neutralizing antibodies to GPCMV and immune response to specific viral glycoprotein complexes (gB, gH/gL/gO, gM/gN, and PC). GP85DISCPC+ virus induced an antibody response similar to that of wild type GPCMV infection in convalescent animals with an average neutralizing antibody (NA) titer of >1:500 in GPL fibroblasts. The average NA titer for GP85DISC animals was lower (1:150). In congenital protection studies, female guinea pigs in each group were vaccinated and mated: GP85DISC (N=14); GP85DISCPC+ (N=19); non-vaccinated (N=15). During late second trimester, animals in all groups were challenged with wild type GPCMV (10^5 pfu). Animals went to term and pup outcome including viral load in target organs were analyzed. Live pup births in GP85DISC (94.4%, $P=0.0002$) and GP85DISCPC+ (96.8%, $P=0.0001$) were significantly higher compared to non-vaccinated animals (63.6%) suggesting that both vaccine strategies were successful. cCMV rate was significantly reduced in GP85DISC vaccinated pups (37%) compared to non-vaccinated group (76%; $P=0.0034$). However, inclusion of the PC in the GP85DISCPC+ vaccine reduced the transmission rate to 0% ($P=0.0001$ compared to GP85DISC or non-vaccinated groups). Overall, both DISC vaccines induced an immune response and decreased the rate of congenital infection but GPCMVPC+ was fully protective, potentially by increasing neutralizing antibody titer on epithelial cells. The increased safety of a non-replication competent virus makes this approach attractive as a CMV vaccine strategy.

530 - Session D.02: Treatment & Prevention

Date: 04/05/2017

Time: 10:55 - 12:20 hrs

Keynote lecture

D-KEY-001

A CMV Vaccine Can Be Made Now

Stanley Plotkin

Univ of Pennsylvania and Vaxconsult, DOYLESTOWN, United States of America

A CMV vaccine would be aimed at three populations: women likely to have children in the future, toddlers who disseminate cytomegalovirus, and transplant recipients. For these targets antibody responses to gB and the pentamer, plus T cell responses (both CD4+ and CD8+) are needed. The main targets of the T cells are pp65 and IE1, but CD4+ help to antibody is crucial. The importance of antibody or T cell responses vary with the population vaccinated, but an effective vaccine should elicit all or most of those responses in order to affect CMV acquisition, passage of the virus to the placenta, and replication in the host.