2008 Congenital Cytomegalovirus Conference

Public Health Action Towards Awareness, Prevention, and Treatment

November 5-7

www.cmvconference2008.com
Sponsor recognition

The 2008 Congenital Cytomegalovirus Conference is sponsored* by:

Centers for Disease Control and Prevention
Congenital Cytomegalovirus Foundation

The Congenital Cytomegalovirus Foundation has received unrestricted general support from:

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National Institute on Deafness and Other Communication Disorders
Radim Diagnostics
Sanofi Pasteur
Virusys Corporation

* No commercial interests were involved in the conference planning effort and no commercial funds or grants were received to specifically support this conference.
Welcome to the CDC in Atlanta, Georgia, the host of the 2008 Congenital Cytomegalovirus (CMV) Conference!

The 2008 Conference represents the largest gathering ever of clinicians and researchers focused on the prevention and control of congenital CMV disease. This truly interdisciplinary Conference includes pediatricians, obstetrician/gynecologists, epidemiologists, virologists, immunologists, vaccinologists, as well as experts in laboratory diagnostics, newborn screening, health promotion, and advocacy. Participants hail from over 20 countries and nearly 30 U.S. states. We applaud your efforts to fight this prevalent and serious disease.

The 2008 Conference continues the tradition started by the first Congenital CMV Conference, held in Orvieto, Italy in 2006. The theme of the 2008 Conference is “Public Health Action Towards Awareness, Prevention, and Treatment”. The purpose is to bring together researchers and clinicians from various fields to discuss the latest research on congenital CMV infection and how these findings can be translated into public health action to improve outcomes among women and children.

Significant advances have been made in congenital CMV research, including a better understanding of virus transmission and disease outcomes, promising treatments for pregnant women and infected newborns, and novel vaccine candidates. We are confident that this Conference will generate new ideas and better implementation of existing ideas.

An exciting feature of the 2008 Conference is a parallel program for families with children affected by congenital CMV. Planned for Thursday, November 7th, this program includes talks by family members and specialists in the fields of congenital CMV research and clinical care, as well as experts in fundraising and advocacy. We are pleased to offer this opportunity for knowledge-sharing and networking among families and the researcher community.

Despite the high disease burden of congenital CMV, prevention and control efforts have faced the persistent challenge of low public awareness. A highlight of the Conference is a lunchtime forum on Thursday, November 7th, where two renowned experts will discuss the role of advocacy in the prevention of childhood diseases and disabilities: Dr. Godfrey Oakley, instrumental in the introduction of folic acid supplementation for the prevention of neural tube defects, and Dr. Jennifer Howse, the President of the March of Dimes Foundation, which has played a major role in improving children’s health in the U.S. We encourage all to attend this special forum.

Thank you for participating in the 2008 Congenital CMV Conference. We hope you have a wonderful experience that sets the stage for the 2010 conference!

Sincerely,

Michael Cannon & Lenore Pereira
Co-organizers
This meeting is dedicated to the memory of Charles Alford, a leader in CMV research and a foremost authority on congenital CMV infection for over 20 years until he retired from the University of Alabama in Birmingham as Emeritus Professor of Pediatrics in 1994. Much of what is known about the natural history and pathogenesis of congenital CMV infection can be traced directly to the work of Alford and the team of investigators he assembled in Birmingham. A graduate of the University Of Alabama School Of Medicine and a pediatrician, Alford trained in virology with Dr. Tom Weller at the Harvard School of Public Health in the early 1960s, studying rubella virus and congenital rubella infection. He used the laboratory to study placental and fetal infections and made key contributions to knowledge of pathogenesis and diagnosis of congenital rubella infection. More importantly, his experiences in Weller’s laboratory provided him with the tools for broader studies of fetal infections and convinced him of the importance of the laboratory in solving clinical problems, a conviction that shaped his career as an investigator and as a mentor. After leaving Weller’s laboratory, Charlie began large scale investigations using the fetal immune response to diagnose and then to screen for congenital infections. Through this work he made important contributions on congenital syphilis and congenital toxoplasmosis but quickly realized that congenital CMV infection was far more common than any other congenital infection and appeared to be a common cause of hearing loss and mental retardation. In the 1970s Charlie assembled a team of investigators at UAB and set about defining the epidemiology, natural history and virological and immunological characteristics of maternal and congenital CMV infection. Charlie’s work did a great deal to convince the scientific and public health community of the importance of congenital CMV infection. This work is defined by 62 publications on cytomegalovirus infection and it is amplified by the fact that he trained a number of investigators who have continued research on congenital CMV infection in the U.S. and in Europe. Remarkably at the same time Charlie was leading his CMV team he led groundbreaking studies of antiviral treatment of neonatal herpes simplex virus infection and herpes encephalitis.

Those who had the good fortune to know Charlie or the better fortune to have worked with him will remember him for his keen intellect and his unwavering commitment to the scientific method as well as his generosity and kindness towards colleagues, trainees and friends. In many ways his work paved the way for this meeting on congenital CMV infection and knowingly or unknowingly we honor him as we continue to study CMV and share our findings.
Co-organizers:

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Scientific advisory committee:

Stuart P. Adler, M.D., Ph.D. (Medical College of Virginia)
Michael J. Cannon, Ph.D. (Centers for Disease Control and Prevention)
Gail Demmler, M.D. (Baylor College of Medicine)
Scott Grosse, Ph.D. (Centers for Disease Control and Prevention)
Gerhard Jahn, M.D. (Universität Tübingen)
Arnaud Marchant, M.D. (University Libre de Bruxelles)
Edward S. Mocarski M.D., Ph.D. (Emory University)
Robert Pass, M.D. (University of Alabama)
Lenore Pereira, Ph.D. (University of California San Francisco)
Stanley A. Plotkin, M.D. (University of Pennsylvania, Sanofi Pasteur)
Maria Grazia Revello, M.D. (Policlinico San Matteo, Pavia)
Yves Ville, M.D. (Université Versailles St. Quentin)

Scientific session moderators:

EPIDEMIOLOGY
Michael J. Cannon (CDC)
Robert F. Pass (University of Alabama at Birmingham and The Children’s Hospital of Alabama)

PATHOGENESIS AND IMMUNOLOGY
Gerhard Jahn (University of Tübingen)
Lenore Pereira (University of California San Francisco)

AWARENESS/BEHAVIORAL INTERVENTIONS
Angie Colson (CDC)
Danielle S. Ross (CDC)

VACCINES
Stanley A. Plotkin (Sanofi Pasteur and University of Pennsylvania)
Mark R. Schleiss (University of Minnesota)

PRENATAL DIAGNOSIS, PROGNOSTIC INDICATORS, CORRELATES OF IMMUNITY, AND TREATMENT
Maria Grazia Revello (Fondazione IRCCS Policlinico San Matteo)
Yves Ville (CHI Poissy Université Paris-Ile-de-France-Ouest)

NEWBORN SCREENING
Sheila C. Dollard (CDC)
Scott D. Grosse (CDC)

POSTNATAL TREATMENT AND FOLLOW-UP
Gail J. Demmler (Baylor College of Medicine)
David W. Kimberlin (University of Alabama at Birmingham)

Family session moderator:

Gail J. Demmler (Baylor College of Medicine)

Conference planner:

Angela S. Fazah (Maximum Technology Corporation)
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Conference Venue

All Conference proceedings will take place at the CDC's Tom Harkin Global Communications Center, with the exception of the Gala Banquet that will take place at the Georgia Aquarium. Scientific sessions will be held in Auditorium A and the Family Sessions will be held in Auditorium B. The Wednesday evening reception will be held in the Global Health Odyssey Museum, which is part of the Tom Harkin Global Communications Center. The Thursday lunch forum will be held in Auditorium B.

CDC Security—Visiting the Global Communications Center

Security

- Government-issued photo ID is required for entry.
- U.S. Citizens must show any government-issued picture ID (i.e., driver’s license, passport, non-driver’s official state ID, certain military ID)
- Non-U.S. Citizens must show a valid passport for access to CDC facilities

Visitors and their belongings are required to pass through a metal detector screening prior to entry. Non-CDC conference attendees are required to remain in the Global Communications Center building once they arrive; visiting other CDC campus buildings is not allowed unless escorted by a CDC staffer. Long-term visitors who have been fingerprinted and have been granted a picture ID badge do not need to be escorted in general access areas. An escorted visitor must carry a valid ID and sign-in at all applicable visitor logs (those at the entrances to the facilities to which the visitor will gain access).

All attendees are subject to a security clearance unless they are CDC employees or contractors.

Important Note: It takes up to 10 business days to process the security clearance for non-U.S. citizens and therefore there is no on-site registration allowed for non U.S. citizens.

Receiving a Visitor’s Badge

Visitor’s badges will be distributed just past the security desk inside the visitor’s entrance of the Global Communication Center (at the front entry of the CDC). Attendees will not be allowed into the meeting room area without a badge.

Parking/Security Car Inspection

Visitors parking is available, yet limited. Attendees traveling by car must pass through a mandatory security car inspection at the security guard station. Security officers will ask to see your picture ID (driver’s license or passport) and will do a quick scan of the undercarriage of your vehicle. Security officers will then ask you to open both the trunk and the hood of your car for inspection. You will then be directed to the visitor parking deck (straight ahead). When you walk into the Global Communications Center from the parking deck (ground level), you will be entering the visitor’s entrance of the Global Communication Center’s visitor’s entrance.

When registering for the conference, please indicate whether you plan to travel to the CDC by car. A special vehicle placard will be provided to you. The placard will speed the mandatory security car inspection process.

Taxi Service

Taxis are allowed to pick up and drop off in front of the Global Communication Center’s visitor’s entrance.

Phones and Internet Access

Cell phone reception is not always strong inside the building. Phones and computers with internet access are located in the business center outside of Auditorium B between the auditorium and the atrium. Wireless internet access is available through the Global Communications Center.

CDC is a non-smoking campus.
Continuing Education Credit

Physicians, nurses, health educators, and other healthcare professionals:

CE (Continuing Education) credits will be provided. Please take advantage of the free CE credit offered at this conference. Alternately, a certificate of attendance is also available. You must go online to register for CE credit and then, complete the session evaluations. It’s easy!

Come to the Information Desk for information on how to get CE and/or staff assistance. You can access the online system and complete the CE evaluations on site or you can do so after the conference until December 8, 2008.

Visit www.cdc.gov/tceonline to access the online system. Search for this course using the course name or course number which is EV1303. The verification code you will need to access this course is CR268F. If you need additional assistance, you can call 1-800-41-TRAIN or 404-639-1292 or email your question to ce@cdc.gov.

Please note: You must complete the CE process by December 8, 2008 in order to earn credit.

Conference maps

Food Services

Full conference registration includes coffee and snack breaks, the Global Health Museum reception on Wednesday evening, and breakfast and lunch on Thursday and Friday. The Gala banquet at the GA Aquarium is not included and must be purchased separately.

One-day registration for Wednesday includes coffee and snack breaks and the Global Health Museum reception that evening. One-day registration for Thursday or Friday includes coffee and snack breaks, breakfast, and lunch. The Gala banquet at the GA Aquarium is not included and must be purchased separately.

All daytime meals will be served in the Global Communications Center atrium, with the exception of the Thursday lunch forum, which will be held in Auditorium B.
Hotel Information

Special rates have been negotiated with two hotels near the meeting site. Reservations should be made by contacting the hotel directly.

Grand Hyatt Atlanta in Buckhead

3300 Peachtree Road NE
Atlanta, Georgia, 30305
Tel: 1-404-237-1234

Reservation details:
- Single/double room rate: $199/night (plus 12% taxes)
- Reservations deadline to receive the conference room rate: October 4, 2008.
- Room block name (to be provided at time of reservation to receive the negotiated rate): CDC CMV International Conference
- Location: Approximately 5 miles from CDC campus.

The Emory Inn

1641 Clifton Road
Atlanta, GA 30329
Tel: 404.712.6700

Reservation details:
- Single/double room rate: $129/night (plus 12% taxes)
- Reservations deadline to receive the conference room rate: October 6, 2008.
- Room block name (to be provided at time of reservation to receive the negotiated rate): CDC CMV International Conference
- Location: Across the street from the CDC campus, adjacent to the Emory Conference Center Hotel. Within walking distance from the CDC (0.2 miles).

Additional Hotels

Additional hotel options without a negotiated group rate are listed below. These hotels are located within 1 to 5 miles of the CDC campus.

<table>
<thead>
<tr>
<th>Hotel Name</th>
<th>Address</th>
<th>Phone Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emory Conference Center Hotel</td>
<td>1615 Clifton Road, Atlanta, GA 30329</td>
<td>404-712-6000</td>
</tr>
<tr>
<td>Courtyard Atlanta Executive Park/Emory</td>
<td>1236 Executive Park Drive, Atlanta, GA 30329</td>
<td>404-728-0708 or toll-free 1-800-321-2211</td>
</tr>
<tr>
<td>Doubletree Atlanta</td>
<td>2061 North Druid Hills Road, Atlanta, GA 30329</td>
<td>404-321-4174 or 800-222-TREE</td>
</tr>
<tr>
<td>Hampton Inn</td>
<td>1975 North Druid Hills Road, Atlanta, GA 30329</td>
<td>404-320-6600 or toll-free 1-800-426-7866</td>
</tr>
<tr>
<td>Holiday Inn, Decatur</td>
<td>130 Clairmont Avenue, Decatur, GA 30030</td>
<td>404-371-0204 or toll-free 1-800-HOLIDAY</td>
</tr>
<tr>
<td>Hotel Indigo</td>
<td>683 Peachtree Street, NE, Atlanta, GA 30308</td>
<td>404-874-9200</td>
</tr>
<tr>
<td>J.W. Marriott at Lenox</td>
<td>3300 Lenox Road, NE, Atlanta, Georgia 30326</td>
<td>404-262-3344 or toll-free 1-800-228-9290</td>
</tr>
<tr>
<td>The Four Seasons</td>
<td>75 14th Street, NE, Atlanta, GA 30309</td>
<td>404-881-9898 or toll-free 1-800-332-3442</td>
</tr>
<tr>
<td>Residence Inn by Marriott</td>
<td>2960 Piedmont Road, NE, Atlanta, Georgia 30305</td>
<td>404-239-0677 or toll free 1-800-331-3131</td>
</tr>
<tr>
<td>Ritz-Carlton Buckhead</td>
<td>3434 Peachtree Road, NE, Atlanta, GA 30326</td>
<td>404-237-2700 or toll-free 1-800-241-3333</td>
</tr>
<tr>
<td>Sheraton Buckhead</td>
<td>3405 Lenox Road, NE, Atlanta, Georgia 30326</td>
<td>404-261-9250 or toll-free 1-800-325-3535</td>
</tr>
</tbody>
</table>

The conference organizers have not conducted a review of the listed hotels, and no endorsement or recommendation is implied.
Hotel Shuttle Information

Grand Hyatt
A complimentary shuttle between the Grand Hyatt and the CDC will be provided Wednesday late morning, Wednesday evening, Thursday morning, Thursday evening, and Friday morning.

Georgia Aquarium
A complimentary shuttle for the Georgia Aquarium Gala Banquet will be provided to all guests who attend. This shuttle will leave the CDC Thursday afternoon and return guests to their hotels that evening.

Emory Inn
The CDC is across the street from the Emory Inn, which offers a free shuttle service upon request.

Key Contacts

If you have general questions during the conference, please contact:

Angela Fazah (256) 655-5314 or Natalie Greene (678) 977-0034

If you have questions related to the programmatic aspects of the conference, please contact:

Michael J. Cannon
Division of Viral Diseases
National Center for Immunization and Respiratory Diseases
Center for Disease Control and Prevention
Phone: 404-790-8296
Email: mcannon@cdc.gov

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Lost and Found

Inquiries regarding lost or found items should be directed to staff members at the Tom Harkin Global Communications Center. All found items are turned over to security to be placed in the lost and found holding area for the CDC. The lost and found contact number is (404) 639-2888.

Poster Session Information

It is the presenter’s responsibility to bring their poster to the conference. We are unable to accept shipped posters. If shipment is necessary, please contact your hotel to determine if they can receive your poster and hold it for you.

At the conference, each presenter will be assigned a number which corresponds to their display board. The poster room will be opened at 12:00pm EST on Wednesday, November 5, 2008 for presenters to mount their posters. We request that all presenters have their posters displayed no later than 5:30pm EST on Wednesday, November 5, 2008.

The primary poster viewing session will take place Wednesday, November 5, 2008, from 7:30pm – 8:30pm EST. Presenters should be at or near their poster during this hour to address questions and comments from viewers. Posters may remain on display until the end of the conference if the presenter desires. We request that all posters be taken down no later than 4:00pm EST on Friday, November 7, 2008. Any posters not removed by this time will be removed by conference staff.
Registration Information

Gala Event Registration

The Gala Banquet at the Georgia Aquarium on Thursday night, November 6, has a separate fee of $75 and includes transportation from the CDC Global Communications Center and back to the Grand Hyatt and Emory Inn hotels. Attendees may purchase a pass for a guest to attend the event for an additional $75. Guests must be accompanied by a conference attendee.

On-site Registration

On-site registration will take place at the Tho Global Communications Center. On-site registration hours are:

Wednesday, November 5, 11:00 a.m. - 5:30 p.m.
Thursday, November 6, 7:00 a.m. - 5:30 p.m.
Friday, November 7, 7:00 a.m. - 10:00 a.m.

Please note: Due to CDC security clearance procedures, on-site registration is not allowed for non-U.S. citizens.

Registration Fees

<table>
<thead>
<tr>
<th>Registration</th>
<th>$475</th>
</tr>
</thead>
<tbody>
<tr>
<td>Student Registration (valid student ID required)</td>
<td>$250</td>
</tr>
<tr>
<td>Family Registration* (per person)</td>
<td>$250</td>
</tr>
<tr>
<td>One-day Registration</td>
<td>$200</td>
</tr>
<tr>
<td>One-day Student Registration (valid student ID required)</td>
<td>$135</td>
</tr>
<tr>
<td>One-day Family Registration</td>
<td>$135</td>
</tr>
<tr>
<td>Gala Banquet at the Georgia Aquarium (Transportation Incl.)</td>
<td>$75</td>
</tr>
</tbody>
</table>

* "Family Registration" is for persons who have a family member affected by congenital CMV.
Registration for Non-U.S. Citizens

All attendees are subject to a security clearance process unless they are CDC employees or contractors. As part of this process, non-U.S. citizens will be asked to provide their passport number and passport expiration date when registering.

Cancellations

Cancellations: Registrations may be canceled and refunds issued if email cancellation notification is received no later than Wednesday, October 1, 2008. Cancellations received after this date will not be accepted. Telephone cancellations will not be accepted.

Special Needs

If you have special needs, please advise a member of the conference staff.

Transportation

The conference will be convened at the Global Communications Center on the CDC campus, 1600 Clifton Road NE, Atlanta, GA, 30333.

Transportation from Hartsfield-Jackson Atlanta International Airport

For your safety, use only authorized vehicles with the airport decal affixed to the bumper and authorized drivers with airport identification badges.

Airport Shuttle Service

For your convenience, two shuttle service options are listed below. You are encouraged to check with individual shuttle services for rates, payment methods, reservations, schedules and pickup points.

In general, shuttles drop off guests at their designated airline’s curbside check-in and pick up guests at the ground transportation area on the west side of the airport.

Atlanta Superior Shuttle
Tel. 770-457-4794

Provides direct service to the Emory Conference Center Hotel, Emory Inn, and other area hotels. Reservations are strongly encouraged.

Fare: approximately $45 round trip, $25 one way to hotels in the CDC area.

Airport Metro Shuttle
Tel. 404-766-6666

Fare: approximately $45 one way to hotels in the CDC area

Other metro area shuttles are also available, visit http://www.atlanta-airport.com.

Please note the conference organizers cannot assume liability for the services and/or rates provided by the companies listed above.

Emory Shuttle Service

Emory University provides a free shuttle for the Emory area. Please refer to the Emory transportation website at http://transportation.emory.edu/shuttles.html for more information on routes and schedules.

Taxi

A number of taxi services are available at Hartsfield-Jackson Airport. Fares to the CDC area may be approximately $45. Visit http://www.atlanta-airport.com.
MARTA

The Metropolitan Atlanta Rapid Transit Authority (MARTA) provides convenient rail service from Hartsfield-Jackson Atlanta International Airport to many points in Atlanta. The entrance to MARTA’s Airport Station is located inside the western end of the airport’s main terminal, near the baggage claim area. Estimated one-way fare is $1.75. MARTA also offers a variety of visitor’s passes ranging from $8.00 to $13.00. These visitor’s passes are available in 1-day, 2-day, 3-day, or 4-day options and offer unlimited system-wide traveling. These passes can be purchased at all MARTA stations.

To get to the CDC (or Emory Inn):

Take the Northeast rail line toward Doraville. Exit at Lindbergh Center station. From Lindbergh Center station (N6), either take a taxi or bus.

Taxis are available right outside the Lindbergh Center station. The taxi fare to the CDC (or Emory Inn), may be approximately $15 - $20.

To take a bus from Lindbergh Center, take the Route #6 bus (Emory) to the Emory Inn/Emory Conference Center stop. (Ask the driver to announce the stop). Get off on Clifton Road at CDC Parkway and walk down to the security booth.

To get to the Grand Hyatt:

Take the Northbound train towards North Springs and Exit at the Buckhead station (N7). The hotel is one block away on Peachtree Street. You may call the hotel at (404) 237-1234 to request pick up from their courtesy car.

Rental Car

Hartsfield-Jackson Airport offers a variety of car rental agencies.

Driving Directions

For those who choose to drive to the Global Communications Center, plan to arrive early to allow time for your car to be inspected and be advised that visitor parking is limited. Driving directions to the Tom Harkin Global Communications Center are available at http://www.cdc.gov/gcc/exhibit/directions.htm.

Visitors’ parking is available, yet limited. Attendees traveling by car must pass through a mandatory security car inspection at the security guard station. Security officers will ask to see your picture ID (driver’s license or passport) and will do a quick scan of the undercarriage of your vehicle. Security officers will then ask you to open both the truck and the hood of your car for inspection. You will then be directed to the visitor parking deck (straight ahead). When you walk into the Global Communications Center from the parking deck (ground level), you will be entering the Global Health Odyssey lobby.

When registering for the conference, please indicate whether you plan to travel to the CDC by car. A special vehicle placard will be provided to you. The placard will speed the mandatory security car inspection process.

Pedestrian Directions

The Emory Inn is ¼ mile from the CDC. To walk to the CDC, take a right on Clifton Road and follow it to the first stop light (CDC Parkway). Cross Clifton Road and follow the sidewalk to the Global Communications Center which sits on the roundabout in front of the visitor’s parking deck.
Conference Events

Conference Proceedings

All scientific conference sessions will take place in Auditorium A with the exception of the lunch forum on Thursday. The lunch forum will be held in Auditorium B. Auditorium A is the first Auditorium on your right after entering the atrium and Auditorium B is located to the left of Auditorium A. Posters will be displayed in the classrooms located at the end of the atrium.

Concurrent Family Session

A concurrent family session will be held in Auditorium B on Thursday, November 6. This is a unique session geared towards the needs of families who have members with congenital CMV.

Family attendees are welcome to register and attend the scientific sessions and conference events on Wednesday, November 5, and Friday, November 7. Also, the Georgia Aquarium Viewing and Gala Banquet on Thursday evening is open to attendees of all ages.

During the conference sessions, a family break room will be available to parents and children. Individuals are responsible for their own child care arrangements during the conference.

Poster Viewing

Posters will be on display and available for viewing by conference attendees throughout the entire conference and will be organized by topic. Presenters will be available during the poster session on Wednesday, November 5 from 7:30-8:30pm. The poster session will take place in Rooms 245-248 and Rooms 254-257 located at the end of the atrium.
Reception at Global Health Odyssey

A catered reception and poster session will be held at the CDC Global Health Odyssey Museum on the evening of Wednesday, November 5. The CDC Global Health Odyssey Museum is located near the visitor’s entrance. Attendees are invited to explore the Global Health Odyssey Museum. This unique exhibit area features award-winning permanent and changing exhibitions that focus on a variety of public health topics, as well as the history of CDC. The changing exhibit on display will be Outbreak: Plagues that Changed History/The Work of Bryn Barnard. The permanent exhibit follows the story of the CDC through 1976. Highlights of the permanent exhibit include an iron lung and biohazard suits. Topics cover polio, smallpox, natural disasters, tuberculosis, and overall health and well-being.

Georgia Aquarium Gala Banquet

An evening at the Georgia Aquarium will take place on Thursday, November 6. Guests will enjoy a catered banquet and an opportunity to experience the world’s largest aquarium. Transportation from the CDC to the aquarium and back to the Grand Hyatt and Emory Inn hotels will be provided.

** Note: The aquarium event requires a ticket purchase not included in the conference fees. See registration information for details.
Conference Agenda
Meeting at a glance

Wednesday, November 5

1:00pm-3:00pm  Opening Plenary Session: Congenital CMV Disease: Challenges and Solutions
3:00pm-5:35pm  Plenary I: Epidemiology
5:35pm-7:00pm  Plenary II: Postnatal Treatment and Follow-Up
7:00pm-9:00pm  Reception and Poster Session

Thursday, November 6

Concurrent Family Sessions
8:00am-8:30am  Family Session I: Parent Support and CMV Advocacy
8:30am-9:20am  Family Session II: Current CMV Research Initiatives
9:20am-10:00am Family Session III: Fundraising for CMV Awareness and Research
10:00am-11:00am Family Session IV: Raising a Child Born with Congenital CMV
2:00pm-5:00pm  Family Forum: Ask the Experts and Meet the Families

Concurrent Scientific Session
8:00am-11:00am  Pathogenesis and Immunology
11:00am-12:30pm Plenary III: Awareness and Behavioral Interventions
12:30pm-2:15pm  Plenary IV: Advocacy
2:15pm-5:00pm  Plenary V: Prenatal Diagnosis, Prognostic Indicators, Correlates of Immunity, and Treatment
5:00pm-10:00pm  Georgia Aquarium Event: Aquarium Viewing and Gala Banquet

Friday, November 7

8:00am-10:00am  Plenary VI: Vaccines
10:20am-1:45pm  Plenary VII: Newborn screening

Conclusion of 2008 Congenital CMV Conference
Conference Agenda
Daily Programming

Wednesday, November 5

1:00pm-3:00pm  Opening Plenary Session: Congenital CMV Disease: Challenges and Solutions

1:00  Welcome Address

1:10  Organizers' Address
Lenore Pereira, Department of Cell and Tissue Biology, University of California, San Francisco, CA.

1:15  Keynote Address: Public Health Action towards Awareness, Prevention, and Treatment.
Gail Demmler, Department of Pediatrics, Baylor College of Medicine, Houston, TX.

1:55  Meet the Families

2:40  Break

3:00pm-5:35pm  Plenary I: Epidemiology,
Moderators: Michael Cannon, Robert Pass

3:00  Recent epidemiologic results that impact awareness, prevention, and treatment.
Michael Cannon, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

3:20  Building passive and active surveillance systems for congenital CMV.
Karen Fowler, Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL.

3:40  Creating CMV-related treatment registries of pregnant women and infants.
Suzanne Luck, Royal Free and University College Medical School, London, U.K.

4:00  Neuroimaging Abnormalities in Asymptomatic Congenital Cytomegalovirus (ACMV) Infection.
Isabella Iovino, Baylor College of Medicine, Houston, TX.

4:15  Birth Prevalence of Congenital CMV Infection and Disease in a Highly Seroimmune Population.
Marisa M Mussi-Pinhata, University of Sao Paulo, Brazil.

Sheila Dollard, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

4:45  Rapid genotyping of cytomegalovirus envelope glycoproteins from toddler's saliva.
Sophie Alain, Faculté de Médecine de Limoges et Laboratoire de Bactériologie-Virologie, Centre Hospitalier Universitaire de Limoges, France.

5:00  CMV congenital infection in children born to HIV infected mothers over a 10 years period (1993-2004).
Marianne Leruez-Ville, Hospital Necker-Enfants Malades, Paris, France.

5:15  Break
5:35 Benefits and risks of current antiviral treatments for children with congenital CMV.
David Kimberlin, Professor of Pediatrics, Division of Pediatric Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL.

5:50 Hearing loss detection and intervention for children with congenital CMV.
John Eichwald, Early Hearing Detection and Intervention, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA.

6:05 Language, educational, and other non-pharmaceutical interventions for children with congenital CMV.
Ira Adams-Chapman, Neonatal-Perinatal Medicine Division of Neonatology, Emory Children's Center Atlanta, GA.

6:20 CMV genotyping in a peri- and postnatal patient group: Correlation with disease and immunity.
Allison Waters, National Virus Reference Laboratory, Dublin, Ireland.

6:35 Oral ganciclovir for severe cerebropathy due to congenital cytomegalovirus infection.
Giovanni Nigro, Director, Pediatric Department, University of L'Aquila, L'Aquila, Italy.

7:00pm-9:00pm Reception and Poster Session

Join us!

You are invited to join us for a catered reception at CDC’s Global Health Odyssey in the Tom Harkin Global Communications Center. Poster session from 7:30pm-8:30pm
(Presenters will be at their posters during this time)
Thursday, November 6

7:00-8:00am  Breakfast in the atrium.

The agenda for Thursday morning includes two separate, concurrent sessions. Shown below are the agendas for each session.

8:00am-11:00am  Concurrent Family Session  
Family Session Moderator: Gail Demmler.

8:00  Family Session I: Parent Support and Advocacy

Gail Demmler, Department of Pediatrics, Baylor College of Medicine, Houston, TX.

8:05  How CMV parents can share information and experiences with each other.  
Janelle Greenlee

8:15  How parents can raise awareness and educate the public about CMV disease.  
Lisa Saunders

8:25  Q&A Discussion

8:30  Family Session II: Current CMV Research Initiatives

8:30  Clinical trials for treatment of newborns with congenital CMV disease.  
David Kimberlin, University of Alabama at Birmingham, Birmingham, AL.

8:40  Clinical trials for prenatal treatment and prevention of congenital CMV infection and disease.  
Stuart Adler, Medical College of Virginia campus of Virginia Commonwealth University Richmond, VA.

8:50  CMV vaccine trials.  
Robert Pass, University of Alabama at Birmingham and The Children’s Hospital of Alabama, Birmingham, AL.

9:00  Family participation in CMV clinical trials.  
Jennifer Bailey

9:10  Q&A Discussion

9:20  Family Session III: Fundraising for CMV Awareness Research

9:20  Fundraising 101: Family fundraising options and resources.  
Julie Rodgers, CDC Foundation.

9:30  Establishing a charitable foundation that Supports congenital CMV.  
Tracy McGinnis

9:40  Unique fundraising opportunities for CMV Families: How to become involved.  
Chad Blakeman

9:50  Q&A Discussion

10:00  Family Session IV: Raising a Child Born with Congenital CMV

10:00  Management and Resources for Children with Developmental and Sensory Disabilities.  
Daniel Williamson, Department of Pediatrics, Baylor College of Medicine, Houston, TX.

10:15  Advocating for your child’s education.  
Gayle Born, Parkaire Consultants, Inc.
10:25  Raising and loving a child with congenital CMV disease.
Lisa Merillat

10:35  Q&A Discussion

11:00  See Plenary III for continued scheduling for combined Scientific and Family Attendees.

2:00  Family Forum: Ask the Experts and Meet the Families.
Interact with CMV experts and other CMV families in an informal setting

8:00am-11:00am Concurrent Scientific Session: Pathogenesis and Immunology
Moderators: Lenore Pereira, Jahn Gerhard.

8:00  CMV pathogenesis and hypoxia at the uterine-placental interface.
Lenore Pereira, Department of Cell and Tissue Biology, University of California, San Francisco, CA.

8:30  Polyfunctional CMV-specific T cell responses in infants with congenital CMV infection.
Laura Gibson, Pediatric Immunology, University of Massachusetts Medical School, Worcester, MA.

8:45  Congenital CMV Infection is Associated with Angiogenic Imbalance in the Placenta.
Ekaterina Maidji, Department of Cell and Tissue Biology University of California, San Francisco, CA.

9:00  Congenital HCMV Infection Impairs CD4 and CD8 T-Cell Responses to HCMV and Unrelated Antigens.
Sharon F Chen, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA.

9:15  Interactions of HCMV with neural progenitor cells - uncovering clues to congenital CNS pathogenesis.
Elizabeth Fortunato, University of Idaho, Moscow, ID.

9:30  Histological and virological diagnosis of symptomatic congenital CMV infection in fetuses.
Liliana Gabrielli, Operative Unit of Microbiology and Virology, University of Bologna and St. Orsola Malpighi University Hospital, Bologna, Italy.

9:45  The Role of RhCMV ULb1 ORF in the Dissemination of RhCMV to Sites of Viral Shedding.
Peter A. Barry, Center for Comparative Medicine/California National Primate Research Center/ Dept. of Pathology & Laboratory Medicine, UC Davis, Davis, CA.

10:00  Hearing loss in a murine model of CMV infection of the developing brain.
Russell D. Bradford, Dept. of Pediatrics, University of Alabama at Birmingham, Birmingham, AL.

10:15  Late-onset developmental delay due to congenital cytomegalovirus infection, asymptomatic in neonate.
Shin Koyano, Department of Pediatrics, Asahikawa Medical College, Ashikawa, Japan.

10:30  Break

11:00am-12:30pm Plenary III: Awareness and Behavioral Interventions (Combined Scientific and Family Attendees)
Moderators: Danielle Ross, Angela Colson.

11:00  What’s Online About Congenital Cytomegalovirus (CMV): A Content Analysis.
Marcia V. Miller, Centers for Disease Control and Prevention, Atlanta, GA.

11:15  A two-year study on Cytomegalovirus infection during pregnancy in a French hospital.
Christelle Vauloup-Fellous, AP-HP, Service de Microbiologie-Immunologie biologique, Hospital Antoine Béclère, Univ Paris-Sud, Paris, France.
11:30 Roundtable presentation and discussion on congenital CMV awareness.  
Christine Prue, Meredith Goff, Jay Schulkin, Linda Smith.

- What do research, theory, and practice reveal about reaching women, nurse-midwives, obstetrician/gynecologists, and child care providers with a CMV prevention message?
- How can congenital CMV researchers build collaborations with these constituencies?

12:30pm-2:15pm Plenary IV: Advocacy

12:30 Lunch and Forum on Advocacy

1:00 Lessons learned from folic acid and how they can be applied to congenital CMV.  
Godfrey Oakley, Department of Epidemiology, Rollins School of Public Health of Emory University, Atlanta, GA.

1:20 How the March of Dimes has successfully influenced policy to improve children’s health.  
Jennifer L Howse, President, March of Dimes Foundation.

1:40 Q&A Discussion

2:00 Break

2:15pm-5:05pm Plenary V: Prenatal Diagnosis, Prognostic Indicators, Correlates of Immunity, and Treatment  
Moderators: Maria Grazia Revello, Yves Ville.

2:15 The current state of CMV prenatal diagnosis.  
Maria Grazia Revello, Servizio di Virologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

2:35 Findings from CMV hyperimmune globulin treatment trials.  
Stuart Adler. Medical College of Virginia campus of Virginia Commonwealth University Richmond, VA.

2:55 Modeling efficacy of a mAb targeting CMV UL128/130/131: prediction of viral load and mAb affinity.  
Jing Yu, Novartis Institutes for Biomedical Research, Cambridge, MA.

3:10 Efficacy of Cidofovir analogue HDP-CDV in Guinea Pig Models of Congenital Cytomegalovirus Infection.  
Rhonda D. Cardin, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH.

3:25 The Frequency of CMV Exposure Among Women Contemplating Additional Pregnancies.  
Beth C. Marshall, Virginia Commonwealth University Health System, Richmond, VA.

3:40 A Proposed Randomized Controlled Trial of Maternal Hyperimmune Globulin to Prevent Fetal Infection.  
Mara J. Dinsmoor, Evanston Northwestern Healthcare, Evanston, IL.

3:55 Roundtable presentation and discussion on prenatal screening.  
Catherine Donner, Tiziana Lazzarotto, Yves Ville, Dana Wolf, Benjamin Wilfond.

- What are the pros and cons of prenatal CMV screening in your country?
- What would improve your prenatal screening program?
- What are the ethical considerations of prenatal screening?
- How do societal and political views of abortion affect prenatal screening?
Georgia Aquarium Event
Join us from 5:00pm-10:00pm for an evening at the largest aquarium in the world for aquarium viewing and a gala banquet.

Friday, November 7
7:00-8:00am Breakfast in the atrium.

8:00am-10:00am Plenary VI: Vaccines
Moderators: Mark Schleiss, Stanley Plotkin.

8:00 Update on the CMV gB vaccine.
Robert Pass, Departments of Pediatrics and Microbiology University of Alabama at Birmingham, School of Medicine And The Children’s Hospital of Alabama, Birmingham, AL.

8:30 Induction of Pluripotent Immunity following Immunization with a Novel Chimeric Vaccine against CMV.
Rajiv Khanna, Australian Centre for Vaccine Development, Herston, Australia.

8:45 A Live, Attenuated CMV Vaccine Protects at the Placental-Fetal Interface in the Guinea Pig Model.
Ryan Buus, Department of Pediatrics, Center for Infectious Diseases and Microbiology Translational Research, University Of Minnesota, Minneapolis, MN.

9:00 Vaccines strategies for therapeutic as well as prophylactic use in preventing congenital CMV.
Edward Mocarski, Emory University, Atlanta, GA.
9:15  **Roundtable presentations:**

- **Animal models**
  Mark Schleiss, Director, Division of Pediatric Infectious Diseases, American Legion Chair of Pediatrics, Associate Chair for Research, Department of Pediatrics, University of Minnesota, Minneapolis, MN.

- **Vaccine Targets**
  Stanley Plotkin, Executive Advisor to CEO, Sanofi Pasteur Emeritus Professor of Pediatrics, University of Pennsylvania Philadelphia, PA

- **Vaccine Trial Endpoints**
  Paul Griffiths, Centre for Virology, Royal Free and, University College Medical School, London, UK.

10:20am-1:45pm  **Plenary VII: Newborn Screening**

  Moderators: Sheila Dollard, Scott Grosse.

10:20  **U.S. newborn screening policy.**

  Rodney Howell, Professor of Pediatrics, Miller School of Medicine, University of Miami, Miami, FL.

10:40  **Issues related to congenital CMV newborn screening.**

  Scott D Grosse, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA.

11:00  **DECIBEL-study: Congenital Cytomegalovirus infection and newborn hearing screening strategies.**


11:15  **Evaluation of DNA extraction methods using dried blood spots for diagnosing congenital CMV infection.**

  Jutte J.C. de Vries, Leiden University Medical Center, Leiden, Netherlands.

11:30  **PCR Detection of Congenital CMV Infection in Minnesota Infants Failing Newborn Hearing Screening.**

  K. Yeon Choi, University of Minnesota, Department of Pediatrics, Center for Infectious Diseases and Microbiology Translational Research, Minneapolis, MN.

11:45-12:45  **Lunch in the atrium.**

12:45  **Roundtable presentations.**

  Maria Barbi, Suresh Boppana, Sheila Dollard, Naoki Inoue.

  - **What are the technical issues associated with CMV testing of dried blood spots?**

  - **What are the logistical and technical issues associated with alternative specimens?**

1:25  **Roundtable discussion and questions from the audience.**

1:45  **Closing Remarks:**

  **Public Health Action towards Awareness, Prevention, and Treatment-Next Steps.**

  Michael Cannon, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

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**Conclusion of 2008 Congenital CMV Conference**
Oral Abstracts
Epidemiology

O-01 Recent epidemiologic results that impact awareness, prevention, and treatment.
Michael J. Cannon, Centers for Disease Control and Prevention, Atlanta, GA.

Epidemiologic studies of CMV infection and congenital disease can highlight the need for awareness, inform prevention research, and focus treatment efforts. This presentation will describe three areas of recent epidemiologic study at the Centers for Disease Control and Prevention: 1) Surveys of CMV awareness among women and obstetrician/gynecologists; 2) Analyses of CMV seroprevalence data from the U.S. National Health and Nutrition Examination Survey (NHANES) to understand transmission modes on a population level; and 3) Literature reviews of the overall burden of congenital CMV infection and disease and the particular burden due to permanent, bilateral hearing loss. These studies are intended to identify women at high risk for giving birth to children with congenital CMV, clarify what messages need to be communicated about congenital CMV prevention, measure the extent of the disease burden, and establish who is likely to need antiviral treatments or other interventions.

O-02 Building passive and active surveillance systems for congenital CMV infection
Karen B. Fowler, UAB Department of Pediatrics, Birmingham, AB.

Congenital CMV infection remains the most common intrauterine infection in the United States resulting in serious disability in infected newborns. CMV however, is not included in any state's recommended or mandated newborn screening tests. The lack of an easy, rapid, inexpensive testing method for CMV, the need for a vaccine or effective treatment to prevent infection, and the absence of advocacy and policy groups for CMV has diminished enthusiasm in the larger scientific community for developing surveillance programs for congenital CMV infection. Additionally, there has been little public awareness about CMV and the possible adverse outcomes following congenital CMV infection. In recent years, new developments in testing for and treating CMV infections, as well as an emerging interest from the newborn hearing screening community for identification of CMV-related hearing loss, has resulted in a need to reconsider surveillance or screening programs for congenital CMV infection. This presentation will include a discussion of the components that are needed for building passive and active surveillance systems for congenital CMV infection. Also, a review of factors that contribute to varying rates of congenital CMV infection in infant populations will be included and how these population variations could impact the development of a surveillance system. Other challenges for developing surveillance systems for congenital CMV infection will also be discussed.

O-03 Creating CMV-related treatment registries of pregnant women and infants
Suzanne Luck, Royal Free and University College Medical School, London, UK.

The role of antiviral treatment for congenital CMV (cCMV) is currently under debate. Only one randomised controlled trial exists to date documenting the efficacy of intravenous (IV) ganciclovir in preventing hearing deterioration in babies with central nervous system symptoms of disease. The availability of oral valganciclovir has seen case reports of the use of antiviral treatment in babies with less severe disease and for prolonged treatment courses emerging in the literature. Nucleoside analogues are not without their risks in this age group, with acute effects on bone marrow being frequently documented and the theoretical risk of carcinogenicity and decreased spermatogenesis in the longer term. While the results of properly conducted RCT are awaited there is a need to monitor treatment trends, toxicity and establish measures of treatment efficacy in these babies and to have the potential for long-term monitoring in the future. In the UK registries are well established for HIV during pregnancy and the monitoring of their offspring. This has provided valuable epidemiological and treatment-related data on which to base national guidelines. Registries of CMV-infected children have been successfully established in the US and Canada and given much an invaluable insight into the course of CMV disease. Changes in legal and regulatory procedures both in the UK and elsewhere have raised new challenges. We discuss the rationale, challenges, and progress in establishing such databases in 21st century Europe.
O-04  **Neuroimaging Abnormalities in Asymptomatic Congenital Cytomegalovirus (ACMV) Infection.**
Isabella Iovino, W. Daniel Williamson, Carol Griesser, Gail Demmler-Harrison. Baylor College of Medicine, Houston, TX.

**Overview:** Congenital CMV infection affects 1% of newborns, most of whom are asymptomatic (ACMV) at birth. Neurologic abnormalities and outcomes in symptomatic congenital CMV (SCMV) have been well studied. However, the prevalence of neuroanatomical abnormalities and outcomes in ACMV are not well characterized. The aim of this study was to determine the incidence and characterize the neuroimaging abnormalities in ACMV newborns.

**Methods:** 109 newborns with ACMV were identified through a newborn screening program and were classified as ACMV based on positive urine culture for CMV at <3 days of age and normal newborn physical examination. 99/109 ACMV neonates had un-enhanced CT scans performed and were analyzed descriptively by three independent reviewers.

**Results:** 51/99 (52%) of ACMV had abnormal CT scans for age and gestation. The most common abnormalities detected overall were dilated extra cerebral spaces 22/51 (43%) and immature myelination 21/51 (41%). Single CT abnormalities occurred in 24/51 (47%) and included focal brain lucencies (n=2), calcifications (n=3), ventricular dilatation (n=2), dilated extracerebral spaces (n=5), immature myelination (n=6), opacified mastoid air cells (n=2), gray/white matter delineation abnormality (n=2), white matter anomalies (n=1), small cranial vault size (n=1), and subarachnoid space dilatation (n=1). 27/51 (53%) had a combination of two or more of the aforementioned CT abnormalities. The most common combinations of abnormalities were immature myelination with dilated extracerebral spaces 9/27 (33%) and ventricular dilatation with dilated extracerebral spaces 8/27 (29%).

**Conclusions:** A high incidence of neuroimaging abnormalities occurred in ACMV infants. Multiple abnormalities occurred most frequently; most often involving immature myelination and dilated cerebral spaces. Longitudinal studies will determine if specific CT abnormalities observed in ACMV infants are associated with progressive hearing loss or neurocognitive outcomes, and may have implications for the neuropathology of the virus in ACMV.

O-05  **Birth Prevalence of Congenital CMV Infection and Disease in a Highly Seroimmune Population.**
Marisa M Mussi-Pinhata, Aparecida Yulie Yamamoto, Rosangela M Moura Britto, Patricia Frizo, Virginia M Wagatsuma, Myriam Isaac Leandro Campos, Suressh Boppana, William J Britt. University of São Paulo, Ribeirão Preto, Brazil.

**Background:** It has yet to be established whether congenital CMV infection and disease in infants and children represents a public health problem in developing countries in populations with high seroprevalence. Only studies not based on universal screening, and evaluating a small sample of selected groups of infants or using non virologic methods have been reported from Latin America.

**Objectives:** To determine the birth prevalence of congenital CMV infection in a population of high seropositivity, and to describe clinical findings of infected newborns.

**Methods:** Infants consecutively born at two Brazilian maternity hospitals were evaluated for the presence of CMV in urine and/or saliva within the first two weeks of life using a PCR assay (DNA-CMV). Congenital infection was confirmed by virus detection in urine samples. Clinical findings present at birth or during the first 15 days of life were recorded. Other congenital infections were ruled out in infants with clinically symptomatic infections.

**Results:** Congenital CMV infection was confirmed in 87/8047 infants (1.08%; 95%CI: 0.86-1.33). Birth weight (2671g vs. 2993g) and intrauterine growth restriction (25.3% vs. 10.7%) were more frequent in infected than uninfected infants (p<0.001). Seventeen (19.5%; 95%CI: 11.8-29.4) infants had at least one typical finding of congenital infection while 4 (4.6%; CI95%: 1.3-11.3) had 3 or more typical findings revealing systemic CMV disease. Any sensorineural hearing loss was found in 6/49 (12.6%; CI95%: 4.6-24.8) children tested at a median age of 26 months.
Conclusions: A high birth prevalence of congenital CMV infection was detected in this Brazilian population. Furthermore, the incidence of symptomatic congenital infection in infants born to women in this highly seroimmune population was similar to that reported in offspring of women from populations with significantly lower rates of seroimmunity to CMV.

O-06 Prevalence of CMV DNA positivity in dried blood spots from newborns in California in 2004-05.
Sheila Dollard, Martin Kharrazi, Terri Hyde, Suzanne Young, Minal Amin, Michael Cannon. National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Background: Laboratory analyses of dried blood spots (DBS) from state newborn screening programs provide a means to identify newborns with CMV infection. Such population-based measures of CMV birth prevalence can identify children in need of early-intervention, assist congenital CMV prevention efforts, and evaluate the efficacy of future CMV vaccines.

Objectives: Determine prevalence rate of CMV DNA in newborn DBS from a representative sample of California births and evaluate risk factors for infection.

Methods: The study population included all newborns (N=4963) tested by the California Newborn Screening Program on three dates (10/28/2004, 1/4/2005, 4/16/2005). DBS were retrieved from frozen storage and sent to CDC in 2007 for laboratory testing. CMV DNA was detected using Taqman-based PCR. Risk group data were obtained from linked newborn and prenatal screening records and live birth records.

Results: The overall CMV DNA birth prevalence rate was 0.6% (95%CI 0.4-0.9%, N=30). CMV DNA birth prevalence rates were highest for infants born to Black mothers (0.8%) followed by Asians (0.7%), Hispanics (0.7%), and Whites (0.5%), with no significant difference between the groups. In a subgroup of babies born to Hispanic mothers (n=2518), CMV DNA birth prevalence decreased across increasing maternal age decades (p trend=0.06) and was higher for children with no father information on the birth record (2.1% vs. 0.6%, p=0.01). CMV IgM antibody was found in 7 (23%) of the 30 CMV positive newborns. Newborns with low birth weight (<2500 g) and/or born preterm (<37 weeks) had higher CMV DNA birth prevalence rates than those who were born of higher birth weight or at term (1.6% vs. 0.5%, p=0.02 and 1.5% vs. 0.5%, p= 0.06, respectively). Log CMV viral load values were inversely related with birth weight (p=0.09) and length of gestation (p=0.03).

Conclusions: Using a PCR-based assay of newborn DBS, congenital CMV birth prevalence estimates in California’s heterogeneous population were similar to those cited previously in the U.S. Due to limited DNA in DBS, birth prevalence rates are likely underestimated in this study. The presence of IgM was not a reliable marker of congenital CMV infection.

O-07 Rapid genotyping of cytomegalovirus envelope glycoproteins from toddler’s saliva.
Jérôme Grosjean, Sébastien Hantz, Sébastien Cotin, Benoît Marin, Christophe Pasquier, François Denis, Sophie Alain. Faculté de Médecine de Limoges et Laboratoire de Bactériologie-Virologie, Centre Hospitalier Universitaire de Limoges, France.

Background: Cytomegalovirus envelope glycoproteins are major targets of neutralizing antibodies and therefore major components of future vaccines. The genetic polymorphism of envelope proteins encoding genes is used to classify CMV strains and may be implicated in CMV behaviours. Though, genotyping is mostly performed after viral culture or PCR cloning which may result in selection bias.

Objectives: To analyse the diversity of strains circulating in day care centers (DCC) and identify multiple infections we needed a new method for CMV classification adapted to direct typing of hundreds of samples without viral culture or cloning, (of low cost and low time consuming), detecting coinfections and recombinant strains.

Methods: From data bank analysis, we developed a new PCR-RFLP method coupled with capillary electrophoresis fragment detection for gpUL55 (gB), gpUL75 (gH) and gpUL73 (gN) genotyping. To detect gB recombinants, gpUL55 typing was performed within two variable zones, the N terminal extremity and the central zone. 212 toddler’s saliva positive for CMV -79 from an emergency unit (EU) and 133 from 6 DCC’s- and 17 isolates from congenitally infected newborns were tested. Results were controlled by direct sequencing of PCR products, and phylogenic trees were built.
Results: We were able to classify the CMV strains in 11 groups (6 prototypic strains and 5 recombinant) for UL55, 2 for UL75, 4 main types and 3 subtypes for UL73, which distinguishes a maximum of 154 different strains. All genotypes were encountered in most of the DCCs and the EU. In two DCC’s all the children were infected by the same genotype for UL55, UL73 and UL75; 37 children (17%) harbored multiple strains, 28 in DCC’s, 9 in the EU, whereas no coinfection was detected in newborn isolates. Recombinants strains (15%) were observed with reproducible profiles in the different populations and different sampling sites.

Conclusion: This method classify CMV strains more precisely than previous RFLP-based classifications, and could constitute the basis of a new classification, particularly for gpUL55, may be more closely related to CMV behaviour. Easy identification of co-infections and recombinants directly from pathological samples, could help large scale epidemiologic studies.

O-08 CMV congenital infection in children born to HIV infected mothers over a 10 years period (1993-2004).

Background: There are few data available concerning the burden of CMV congenital infection in children born to HIV infected mothers.

Objectives: To evaluate the prevalence and the maternal risk factors for cytomegalovirus (CMV) congenital infection in children born to HIV infected mothers. CMV congenital infection was studied in children born alive between 1993 and 2004 and enrolled in the French Perinatal Cohort (EPF), a national prospective multicenter cohort of mother-to-child HIV transmission.

Methods: CMV congenital infection was screened by rapid culture (1993 to 2001) and rapid culture or real time PCR (since 2001) in a urine sample obtained within the ten first days of life. Maternal CMV serology was recorded in EPF files until 2001 and 91.9% of mothers were seropositive for CMV.

Results: CMV neonatal screening was performed for 5019 of the 7563 newborns included in EPF (1993 to 2004). The overall prevalence of CMV infection was 2.3% (95% CI:1.9-2.8), this prevalence was higher in HIV infected newborns (10.3%; 95% CI: 5.6-17.0) than in HIV uninfected newborns (2.2%, 95% CI:1.8-2.7), p<0.01. Among children term born and HIV uninfected, year of delivery, maternal young age, time of ART introduction and low maternal CD4+ cell count (<200/mm3) near delivery were factors independently associated with congenital CMV infection in a logistic regression. Among CMV infected newborns, 30.8% of those HIV co-infected had CMV related symptoms when only 6.3% of HIV uninfected newborns were symptomatic.

Conclusions: In HIV co-infected infants CMV congenital infection remained highly prevalent over time and associated with high morbidity. Conversely, the prevalence of CMV congenital infection in HIV uninfected infants decreased significantly from over 3.0% to 1.1% in the most recent periods. This 1.1% prevalence was similar to the prevalence expected in a highly immune population of pregnant women. This decrease of CMV congenital infection prevalence in children born to HIV infected mothers was concomitant to the increase of HAART usage in their mothers.

Postnatal Treatment and Follow-Up

O-09 Benefits and risks of current antiviral treatments for children with congenital CMV.
David Kimberlin, University of Alabama at Birmingham, Birmingham, AL.

Congenital cytomegalovirus infection can result in clinically apparent (symptomatic) disease at birth, or in clinically inapparent (asymptomatic) infection. Data on the treatment of congenital CMV are only available for babies in the former group. Among neonates with symptomatic congenital CMV, administration of six weeks of intravenous ganciclovir protects against hearing deterioration over at least the first two years of life, and may lead to improved neurodevelopmental outcomes as well. The dose of pharma-
ceutical-grade valganciclovir which produces similar blood concentrations of ganciclovir as does intrave-
nous ganciclovir has been identified. A new multicenter study being conducted by the NIAID Collabora-
tive Antiviral Study Group is now evaluating whether six months of oral valganciclovir therapy results in
better hearing and neurodevelopmental outcomes than six weeks of oral valganciclovir therapy. The main
toxicity of ganciclovir or valganciclovir is neutropenia; with intravenous ganciclovir and oral valganci-
clovir, 38-68% of treated babies develop neutropenia during a six week course of therapy. In animal models,
ganciclovir is carcinogenic and gonadotoxic, although these toxicities have not been seen in humans. As
additional efficacy data from controlled trials become available, identification of which babies to treat and
for how long will improve. At the current time, though, only symptomatic babies with central nervous
system involvement should be considered for intravenous ganciclovir therapy, and oral valganciclovir
should be reserved for subjects on the multicenter NIAID trial due to the fact that it is not commercially
available.

O-10 Hearing loss detection and intervention for children with congenital CMV.
John Eichwald, Centers for Disease Control and Prevention, Atlanta, GA.

The Early Hearing Detection and Intervention (EHDI) program was established at the Centers for Disease
Control and Prevention (CDC) under the Children’s Health Act of 2000. As part of this legislation, Con-
gress authorized the CDC to identify the causes and risk factors for congenital hearing loss. The EHDI
program has been evaluating the effectiveness of newborn and infant hearing screening, evaluation and
intervention programs and systems conducted by state-based programs. A recent comparison of newborn
hearing screening data reported to survey data collected at school age revealed significant differences be-
tween the birth prevalence of hearing loss identified in the newborn period and the prevalence reported
in later childhood. This presentation will provide an overview of the EHDI program CMV research activi-
ties and the recent prevalence comparisons. Possible reasons for prevalence differences, including late
onset of hearing loss due to CMV will be discussed.

O-11 Language and educational interventions for children with Congenital CMV
Ira Adams-Chapman, Emory University, Atlanta, GA.

In the United States, approximately 40,000 infants are born each year with congenital CMV infection. The
vast majority of these infants have no clinical manifestations of disease at birth; however approximately
20% of infected infants develop permanent neurologic sequelae. The most common neurologic defect is
sensorineural hearing loss (SNHL) which is present up to 65% of infected infants. Others have evidence
of cerebral palsy, motor delay, language delay, cognitive impairment, seizures and visual defects.

The pathophysiology of neurologic injury in Congenital CMV infection is multifactorial. The risk of neu-
rologic sequelae and developmental delay is higher among those infants with overt evidence of CNS in-
jury such as microcephaly, calcifications or seizures and these findings correlate with viral load. Asymp-
tomatic infants have a lower but significant risk for progressive hearing loss and learning difficulties.

Increased severity of disease correlates with the need for developmental therapies. Targeted developmen-
tal therapies to address specific patterns of neurodevelopmental impairment are extremely important.
Timely and appropriate audiological evaluations are needed to ensure that affected children have appro-
priate auditory and neurosensory input during the critical phases of brain development.

Strategies to support the educational needs of affected children include but are not limited to the follow-
ing interventions:

1. Early diagnosis and management of hearing loss
2. Alternative communication devices
3. Speech and language therapy services
4. Physical therapy services
5. Educational support services
O-12  CMV genotyping in a peri- and postnatal patient group: Correlation with disease and immunity.

Background: Cytomegalovirus (CMV) infects a range of cell types and virulence may be linked to genetic variations within the viral genome. In the present study, we tested whether CMV genotypic variation, in the UL144 and glycoprotein B (gB) genes were associated with changes viral load, immune response and clinical outcome. The UL144 gene is found in clinical isolates but lost in laboratory-attenuated strains, implicating a potential role in viral pathogenesis. gB is immunogenic and stimulates antibody responses in the host.

Objectives: We sought to investigate whether the UL144 genotypes and Glycoprotein B genotypes were singularly or in combination associated with changes in viral load, cytokine production and the clinical outcome of CMV infection in peri- and postnatal patients.

Method: Peri- and postnatal CMV patients (< 1yr), identified in Ireland between January 2006 and December 2007 (n=51), were included in the study. The excreted and plasma CMV viral load was determined using the Artus Quantitative real-time CMV kit. Th1- and Th2-type cytokines were examined using multiplex bead arrays and Luminex technology. The UL144 and gB genotypes were determined by PCR and sequencing.

Results: All UL144 genotypes (A, B, C) were detected. UL144 genotypes A and C were positively correlated with viremic CMV infection in peri- and postnatally infected babies (p<0.01). There was no correlation between gB genotype and either the excreted or plasma viral load. IL-10, IL-8 and TNF-α; were significantly upregulated in all infected babies (p<0.0001, p<0.0027 and p<0.0093, respectively). Changes in cytokine levels did not correlate with genotype. IL-10 was significantly upregulated in the viremic babies when compared with non-viremic children.

Conclusions: Disease severity often correlates with CMV plasma viral load and as such increased IL-10, IL-8 and MIP-1α and infection with UL144 genotype C may correlate with patient clinical outcome. This is consistent with previous publications that found genotype C was associated with more severe clinical outcome in congenitally infected babies. This study highlights the possibility that both viral genes and host immune response factors may impact on the pathogenesis of CMV.

O-13  Oral ganciclovir for severe cerebropathy due to congenital cytomegalovirus infection.
G. Nigro, Pediatric Department, University of L’Aquila, Italy.

Antiviral therapy for infants with congenital cytomegalovirus infection: the European experience G. Nigro Pediatric Department, University of L’Aquila, Italy The treatment of infants with congenital CMV infection is based on the use of ganciclovir, which has been given at different regimens and for different clinical manifestations. A few case reports or case series and one study have been published. In 12 infants with severe neurological manifestations, a pilot study (Nigro G et al, J Pediatr 1994) showed a better virological and clinical outcome after a long regimen treatment (7.5 mg/kg twice daily for 2 weeks, followed by 10 mg/kg three times a week for 3 months) than a short and low-dosage regimen (5 mg/kg twice daily for two weeks). Another pilot study (Galli L et al, Pediatr Infect Dis J 2007) on plasma concentration in newborns and infants treated with valganciclovir suggested doses of 15 mg/kg given twice daily. The favourable effects of ganciclovir in the prevention of sensorineural hearing loss in children with asymptomatic congenital CMV infection were recently shown by Lackner A et al (J Laryngol Otol, 2008). Preliminary data are reported on 44 congenitally-infected infants with severe cerebropathy (microcephaly, calcifications, dysplasia, leukomalacia, neurisensorial hearing loss, chorioretinitis), as shown by clinical, ultrasound and CT scan/magnetic resonance images. Oral ganciclovir was given to 15 infants for 5 to 28 months, and follow-up lasted 3 to 7 years (mean: 4.9), 16 infants received iv ganciclovir, and 13 were not treated. Psycho-motor development was within normal limits in 8 (53.3%) orally-treated infants, compared to 2 iv-treated infant (12.5%) and none of the untreated infants (p<0.001). Normal hearing was shown in 5 orally-treated infants (33.3%), compared to 5 iv-treated infant (31.2%) and 1 of the untreated infants (5.8%). Both oral and iv ganciclovir was generally well tolerated. In conclusion, a longer duration of ganciclovir therapy has been associated with favourable outcome more frequently than shorter cours-
es, probably related to the longer inhibition of CMV. In fact, this virus reactivates soon after stopping therapy, and some of the favorable results obtained during ganciclovir therapy may be lost. Prolonged oral ganciclovir treatment was associated with a more favourable neurological but not auditory outcome than iv ganciclovir.

Pathogenesis and Immunology

**O-14** CMV Pathogenesis and hypoxia at the uterine-placental interface.
Lenore Pereira, Department of Cell and Tissue Biology University of California, San Francisco, CA.

**Background:** CMV is the leading cause of congenital viral infection with an incidence in the United States of 1-3% of live births. Primary maternal CMV infection during gestation poses a 40% risk of intrauterine transmission in contrast to recurrent infection. Symptomatic infants may die in the neonatal period and most survivors have permanent sequelae, including mental retardation, deafness and retinitis. Our studies revealed that CMV spreads from sites of infection in uterine arteries to invasive cytotrophoblasts, then to villi floating in maternal blood. The neonatal Fc receptor for IgG transcytoses immune complexes with virions that replicate in cytotrophoblasts expressing CMV receptors. Anti-CMV IgG with low neutralizing titer enables infection that spreads to stromal fibroblasts and the fetal vasculature. Women with strong humoral immunity contain neutralizing IgG and virion gB in syncytiotrophoblasts without virus replication. These studies establish a central role for humoral immunity in suppression of CMV infection at the uterine-placental interface.

**Objectives:** It was recently reported that women with primary CMV infection, treated at midgestation with intravenous hyperimmune globulin (HIG) with high-avidity to CMV gB, significantly reduce symptomatic congenital disease. We engaged in a collaborative study to examine placental pathology, viral replication and molecular changes that could offer an explanation for the efficacy of intravenous HIG treatment.

**Methods and Results:** Histological analysis of biopsy specimens from infected placentas showed a variety of floating villi damage with inflammation, villous fibrosis and necrosis. Immunostaining revealed expression of proteins associated with hypoxia in women who smoke and those with the pregnancy disorder preeclampsia. In contrast, HIG-treated placentas developed numerous small vascularized villi suggesting compensation for hypoxia. These results suggest direct viral damage resulting in placental hypoxia. Compensation of the placenta in a hypoxic environment promotes villous regeneration especially pronounced with early HIG treatment.

**Conclusions:** Current models illustrate our knowledge of focal CMV replication that results in cellular injury associated with long-term effects on placental development and compensatory remodeling after HIG treatment.

**O-15** Polyfunctional CMV-specific T cell responses in infants with congenital CMV infection.
Laura Gibson, Don Diamond, Tumul Srivastava, Ravindra Rawal, Katherine Luzuriaga. Pediatric Immunology, University of Massachusetts Medical School, Worcester, MA.

**Background:** Better understanding of neonatal antiviral cell-mediated immune responses is crucial to the design of CMV vaccine strategies to reduce vertical CMV transmission or severity of congenital infection. T cells capable of multiple anti-viral effector functions (polyfunctional T cells) may be associated with protection from severe viral infection.

**Objectives:** To characterize the functional properties of CMV-specific CD4+ or CD8+ T cell responses in infants with congenital CMV infection.

**Methods:** The study cohort includes 15 infants (13 congenital and 2 postnatal) and 8 adults (4 chronic and 4 primary during pregnancy) with CMV infection. Peripheral blood mononuclear cells were incubated with pools of overlapping peptides spanning CMV pp65 or immediate early (IE)-2 protein for 6 hours. Anti-viral T cell functions were measured by cytokine secretion (IFN-γ, TNF-α, or IL-2), chemokine secretion (MIP-1β), or degranulation (CD107a/b).
**Results:** CMV pp65-specific CD4+ or CD8+ T cell responses were detected in 3 of 4 infants studied at frequencies of 0.03 - 0.5% of CD4+ or CD8+ T cells. In all 3 infants, CMV-specific T cells expressed 1 or 2 functions, but the pattern of responses differed between infants. In a mother-infant pair, responses against pp65 were detectable by single- or bi-functional CD107a/b, MIP-1β, TNF-α, and IFN-γ (not IL2) expression by both CD4+ and CD8+ T cells in the mother at 27 weeks gestation (9 weeks after onset of primary CMV infection), but only by CD8+ T cells in her infant at 2 days of age. No functional CMV-specific CD4+ T cells were detectable in the infant.

**Conclusions:** Preliminary results suggest that in infants with congenital CMV infection, patterns of CMV-specific T cell effector function may vary, polyfunctional T cells are detectable, and the functionality of some T cell subsets may be reduced compared to adults. Studies further characterizing T cell responses to CMV pp65 or IE-2 proteins are ongoing for the remainder of the study cohort.

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**O-16 Congenital CMV Infection is Associated with Angiogenic Imbalance in the Placenta.**

**Background:** Human cytomegalovirus (CMV) is a leading cause of congenital viral infection accounting 1-3% of all live births in the US. Different strategies - evaluation of maternal humoral immunity and detection of viral DNA in amniotic fluid - have been used for prenatal diagnosis of congenital infection. Availability of preventative treatment provides a solid rational for development of early biomarkers.

**Objectives:** Detailed immunohistological analysis of congenitally infected placentas with and without hyperimmune globulin (HIG) treatment (Nigro et al., NEJM 2005) showed that untreated infection injures the uterine vasculature and fetal capillaries that could restrict blood flow causing local hypoxia. Up-regulation of hypoxia-inducible factors, vascular endothelial growth factor (VEGF) and its inhibitory receptor soluble Flt1 (sFlt1) in congenitally infected placentas suggested circulating factors could serve as suplementary evidence of viral replication in specialized cells of the placenta.

**Methods:** Amniotic fluid (57 samples) from 3 groups was studied: CMV infected-HIG-treated prevention, infected untreated, and healthy seronegative controls. Concentrations of sFlt1, VEGF bound to sFlt1 (bVEGF), “free” VEGF (active form), and placental growth factor (PlGF) were measured using commercial ELISA kits (R&D System and Chemicon International).

**Results:** Amniotic fluid from congenital infection contained exceptionally high levels of sFlt1 and bVEGF, but little PlGF and no free VEGF. In the HIG-treated prevention group, sFlt1 and bVEGF levels were sharply reduced, and PlGF was unchanged. In addition, the anti-angiogenic index (sFlt1/PlGF) was significantly elevated in amniotic fluid in all congenital infection as compared with the healthy control group (P<0.001). In contrast, the anti-angiogenic index was significantly lower with HIG prevention (P=0.037) as compared with untreated infection.

**Conclusions:** Our study identified promising biomarkers of CMV infection at the uterine-placental interface that correlate with dysregulated placental functions and viral transmission. Combined quantification of maternal IgG avidity, anti-angiogenic index and detection viral DNA in amniotic fluid could improve diagnosis of pregnancies endangered by congenital infection and disease.

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**O-17 Congenital HCMV Infection Impairs CD4 and CD8 T-Cell Responses to HCMV and Unrelated Antigens.**
Sharon F Chen, DB Lewis, TH Holmes, TR Slifer, V Ramachandran, L-X Liu, AM Arvin, CL Dekker. Department of Pediatrics, Stanford University School of Medicine, Stanford, CA.

The post-natal αβ-T-cell response in congenital HCMV infection is poorly understood. We prospectively evaluated responses in 46 congenitally-infected infants identified by prospectively screening infants in Northern California. Blood samples obtained at 4, 12 and 36 mo of age were compared with samples from age-matched children with postnatal HCMV infection identified in a separate study. We performed intracellular cytokine assays of T cells in whole blood stimulated with 1) a lysate of HCMV-infected
human embryonic fibroblasts (HCMV), 2) a pool of overlapping peptides of pp65 tegument protein (pp65), 3) staphylococcus enterotoxin B (SEB), that served as a positive control and a means to assess non-HCMV-specific immunity, and 4) lysate of mock-infected fibroblasts (negative control). CD4 and CD8 T-cell phenotype were determined by co-expression of CD69 and intracellular production of IFN-γ, with frequencies expressed as means [95% CI]. CD4 and CD8 T-cell responses to HCMV and pp65 stimulation were low in the congenital group but comparable to the postnatal group at all time points tested. However, congenitally-infected infants had a significantly lower CD4 T-cell response to SEB compared to the postnatal group (congenital 0.23 [0.18,0.30] vs. postnatal 0.76 [0.50,1.15] at wk 30; p≤0.05). We evaluated 6 symptomatic and 40 asymptomatic congenitally infected infants and found that HCMV- and pp65-specific CD4 T-cell responses were comparable at all time points. In contrast, HCMV-specific CD8 T-cell responses were significantly lower in symptomatics than asymptomatics at wk 60 (symptomatic 0.01 [0.00,0.03] vs. asymptomatic 0.05 [0.02,0.09]; p<0.05). Furthermore, symptomatic infants appeared to have an extensive CD8 T-cell defect as SEB response was significantly lower compared to asymptomatic infants at wk 30 (symptomatic 0.58 [0.32,1.06] vs. asymptomatic 1.32 [0.97,1.81] p<0.05). Conclusions: In the first year, infants with congenital HCMV infection demonstrate a generalized impairment of CD4 T-cell antigen response as demonstrated by a significantly lower response to SEB compared with postnally-infected infants. Furthermore, development of CD8 T-cell responses appears to be globally impaired in the congenitally-infected symptomatic infants. We speculate that a generalized deficient αβ-T-cell response in infants with congenital HCMV infection during the first year of life may adversely impact their immune response to routine childhood immunizations.

O-18 Interactions of HCMV with neural progenitor cells - uncovering clues to congenital CNS pathogenesis.
Elizabeth Fortunato, Min Hua Luo, Philip H. Schwartz, Holger Hannemann. University of Idaho, Moscow, ID.

Background: Although HCMV has a wide range of permissiveness in vivo, the fetal brain is the main site of the drastic manifestations of HCMV infection. It has been suggested that the severity of the neuropathological changes and clinical outcome may be associated with the stage of CNS development at which congenital infection occurs, yet the mechanism of HCMV pathogenesis in the developing CNS remains poorly understood.

Objective: Our objective is to translate the information we gain from studying human cytomegalovirus (HCMV) infection of clinically relevant neural progenitor cells (NPCs) and their derivatives in vitro into an understanding of the development of central nervous system (CNS) defects in congenitally infected infants. We will also draw upon our earlier studies of the interaction of HCMV with the DNA repair machinery of the permissively infected fibroblast, which showed that DNA damage responses were not carried to completion. This had consequences for the host cell genome, as exogenously induced damage was removed preferentially from the viral DNA.

Methods: We have utilized a new primary cell system (NPCs) to study the effects of HCMV infection in tissue culture, using both a lab-adapted and clinical isolate of the virus. We have utilized Western blotting, immunofluorescent localization, real time RT-PCR and FISH analyses for these studies.

Results: Our studies in NPCs and their glial and neuronal derivatives find them fully permissive for HCMV infection. However, we also find evidence of long-term expression of viral antigens and release of virions without cell death in a subpopulation of infected neurons. Infection of NPCs also points to perturbation of stem cell marker expression and a propensity to drive them to differentiate. We have also determined that HCMV induces site specific damage on chromosome 1 in NPCs and astroglia. Fine mapping of these sites has revealed links to the development of hearing loss in infected infants. Importantly, analysis of the chromosome 1 breaksite-associated genes in both fibroblasts and NPCs show downregulation of gene expression during infection.

Conclusions: Study of infection of NPCs is a viable new tissue culture system in which to dissect the interplay between HCMV and its host cell.
**O-19** Histological and virological diagnosis of symptomatic congenital CMV infection in fetuses.
Liliana Gabrielli, Stefania Lega, Maria Pia Foschini, Donatella Santini, Marcello Lanari, Brunella Guerra, Giulia Piccirilli, Tiziana Lazzarotto. Operative Unit of Microbiology and Virology, University of Bologna and St. Orsola Malpighi University Hospital, Bologna, Italy.

**Background:** Congenital CMV infection is a major cause of central nervous system damage leading to sensorineural hearing loss, mental retardation and cerebral palsy.

**Objectives:** Due to the scarcity of information, we studied fetal CMV infection to describe the type of organ involvement and histomorphological findings, and identify histological and virological markers for an adverse outcome.

**Methods:** 30 cases of fetal congenital CMV infection documented by prenatal diagnosis (amniotic fluid CMV culture positive and PCR positive) were studied. At the time of amniocentesis, abnormal ultrasonographic findings had been recorded in 9 of the 30 fetuses (30%). Two fetuses died in utero. The remaining pregnancies were electively terminated at 22 weeks gestation. Macroscopic and histomorphological examinations were performed and CMV early (ppUL44) and late (major protein 55KD) antigen expression in the tissues of different organs were studied using immunohistochemical staining procedures.

**Results:** Histological examination of all placentas showed varying degrees of chronic villitis and early and late CMV antigens were detected by immunohistochemistry. Fetal organs positive for early CMV antigens were as follows: pancreas (95%), lung (88%), kidney (83%), liver (68%), brain (52%) and heart (27%). Severe inflammatory cell reaction suggestive of histological damage was found in only 33% of the brains studied and in only 4% of each of the remaining organs. Severe inflammatory cell infiltration was frequently associated with pathological findings at macroscopic observation (hydrocephalus, cerebellar hypoplasia, etc.) and/or at ultrasonography (cerebral ventriculomegaly, hyperechogenic bowel, etc.). A correlation was also found between the presence of late CMV antigens and organ damage, but the study is still underway.

**Conclusions:** The presence of early CMV antigens in fetal organs is correlated with the dissemination of infection, but does not indicate organ damage. Instead, the presence of a severe inflammatory cell reaction is highly suggestive of CMV symptomatic infection, especially when it is associated with abnormal macroscopic or ultrasonographic findings and probably also with the presence of late CMV antigens. Using these parameters, the percentage of organ damage observed in fetuses is the same as those observed in newborns with CMV congenital infection.

**O-20** The Role of RhCMV ULb1ORF in the Dissemination of RhCMV to Sites of Viral Shedding.

Rhesus cytomegalovirus (RhCMV) is ubiquitous in both free-ranging and colony-reared populations of rhesus macaques. Clinical signs of RhCMV infection are almost never observed in either population following either natural exposure to wild-type virus (RhCMVWT) or experimental inoculation with the 68-1 strain of virus (RhCMV68-1). A hallmark of natural infection with RhCMVWT is that a large percentage of infected macaques (~75%) shed virus in bodily fluids for a sustained period of time. This stands in contrast to our observations with RhCMV68-1 in which only low titer shedding in fluids such as saliva or urine, is sporadically observed, or often not detected. These RhCMV strains are distinguished by differences in the coding content of the ULb’ region, such that RhCMV68-1 lacks UL128, UL130, and three alpha chemokine-like ORF found in RhCMVWT (Oxford et al, Virology, 373:181, 2008). To determine whether these genetic differences account for the differences in shedding potential between RhCMVWT and RhCMV68-1, an initial study was performed in which 2 macaques were inoculated with RhCMV68-1 and two RhCMV variants containing the full-length ULb’ (RhCMV22659 and RhCMV21252). Unlike previous inoculations with RhCMV68-1, sustained excretion of RhCMV was detected in urine and saliva. Using differential PCR to distinguish between the three variants, only RhCMV22659 and RhCMV21252 were detected in urine and/or saliva, or recovered from parotid gland explanted in
culture. A subsequent study is underway in which groups of macaques have been inoculated with a single variant. Sustained excretion of RhCMV in saliva has been observed only with RhCMV22659 and RhCMV21252. These observations implicate the UL128/130/131a complex in mediating transmission from the site of inoculation to sites of shedding and/or maintenance of shedding at mucosal surfaces. These results support observations with HCMV that suggest that this viral protein complex should be a vaccine target to minimize the potential for horizontal, and potentially vertical, transmission of virus. In addition, a biopsy of the inoculation sites detected the presence of polymorphonuclear leukocytes following inoculation with RhCMV22659 and RhCMV21252, but not with RhCMV68-1, suggesting a functional role for the alpha chemokines encoded within ULb'.

O-21 Hearing loss in a murine model of CMV infection of the developing brain.
Russell D. Bradford, Glenn Robert B. Bantug, Stipan Jonic, William J Britt. Dept. of Pediatrics, University of Alabama at Birmingham, Birmingham, AL.

Background: Congenital cytomegalovirus (CMV) infection represents the most common infectious cause of hearing loss in the developed world. Little, however, is known about the pathogenesis of CMV-associated hearing loss. In a recently described murine model of CMV infection of the developing central nervous system (CNS), neonatal mice are infected with a non-lethal inoculum by intraperitoneal (i.p.) injection. Mice first develop systemic infection, with subsequent dissemination to the CNS. This model has demonstrated usefulness in the study of the pathogenesis of CMV infection of the developing brain, but hearing outcomes are unknown.

Objective: To test the hypothesis that mice with CNS infection of the developing brain with MCMV will demonstrate hearing loss.

Design/Methods: Litters of BALB/c mice were infected i.p. at birth with 200 pfu MCMV. Age-matched uninfected litters were maintained as controls. Mice were weaned at 6 weeks of age. At 3 months of age, 12 infected and 12 uninfected mice were sedated (ketamine 100 mg/kg and xylazine 10 mg/kg), and auditory brainstem response testing (ABR, click stimulus, 90 dB to 10 dB, in -10 dB steps) was performed on each ear to determine sound pressure level (SPL, in dB) thresholds.

Results: An interpretable ABR was obtained from 22 of 24 ears in uninfected mice and 21 of 24 ears of infected animals. All ears tested from uninfected animals showed normal hearing (SPL threshold ≤40 dB). 48% of ears from MCMV infected animals were abnormal (SPL threshold ≥50 dB). Mean SPL threshold among mice neonatally infected with MCMV was 44.3 dB (range 30-70 dB, median 50 dB) compared to 26.4 dB (range 20-40 dB, median 30 dB) in uninfected mice.

Conclusions: Our model has previously been used to study the pathogenesis of CMV infection of the developing CNS. We now demonstrate that these animals also develop hearing loss, with a 20 dB change in mean and median SPL threshold from age-matched control animals. Further study of the pathogenesis of hearing loss in these animals could provide insight into mechanisms leading to hearing loss in children with congenital CMV infection.

O-22 Late-onset developmental delay due to congenital cytomegalovirus infection, asymptomatic in neonate.

Background & Objectives: Cytomegalovirus (CMV) is the most common cause of congenital infection and ascribed to late-onset neurological disorders, such as developmental disability, sensorineural hearing loss (SNHL), cerebral palsy, and epilepsy. Some of the late-onset cases have no clinical symptoms in the newborn periods. Previously, we demonstrated that 15% of severe SNHL was due to congenital CMV infection (Ogawa et al., 2007). This study aims to clarify the impact of congenital CMV infection on developmental disability and to identify clinical characteristics of the identified cases.
**Methods:** Twenty Japanese children with developmental disability (DQ score less than 70) were enrolled. Enrollment was done based on exclusion of cases of known causes, including Down’s Syndrome and other chromosomal abnormalities, genetic defects in metabolisms and hormones, difficult delivery with some complications such as fetal distress, and mother under drug and alcohol influence. Severity of the developmental delay was scored by the Japanese standard method, and hearing capability was evaluated by auditory brain-stem response. Nine of the cases had hearing defects and at least 7 of them experienced >1 episode of seizure. DNA samples were prepared from dried umbilical cord specimens that were available for every individual born in Japan, and both the conventional and real-time PCR assays were used for detection of CMV. Genotypes of glycoprotein B (gB), gN, gH, UL144 genes were analyzed by sequencing.

**Results:** Out of the 20 cases, 5 cases (2 severe, 2 moderate, and 1 mild disability) were CMV positive. None of them had any CMV-associated clinical manifestations at the very early phase of their lives. Their developmental delay appeared relatively later (1-12 months), and some of them have exhibited not only physical but also mental retardation after infancy. Intracranial calcification and hearing defect were observed in 4 and 3 of the 5 cases, respectively. There was no relationship between virus genotype and clinical severity.

**Conclusions:** Significant proportion of developmental disability cases without any known cause is due to congenital CMV infection. Since CMV-associated developmental delay is late-onset and independent from hearing defect, it is critical to detect congenital infection just after birth by screening programs.

### Awareness and Behavioral Interventions

**0-23 What’s Online About Congenital Cytomegalovirus (CMV): A Content Analysis.**
Marcia V. Miller, Danielle S. Ross, Sonja A. Rasmussen. Centers for Disease Control and Prevention, Atlanta, GA.

**Background:** Cytomegalovirus (CMV) infection can cause birth defects and developmental disabilities when a woman becomes infected with it during pregnancy. Although there is no vaccine currently available, prevention is possible by adopting certain hygienic behaviors. However, many women are not aware of CMV or the behaviors that can possibly prevent infection. The internet is a potentially rich resource for this information for women who are pregnant.

**Objectives:** To assess the amount and quality of information available on the internet about the prevention of congenital CMV.

**Methods:** A content analysis of congenital CMV web sites was performed in June of 2008 based on searches using four main “metasearch” engines. A metasearch engine allows a user to enter search criteria once, after which the request is sent to multiple single search engines simultaneously. The web pages resulting from these searches and the linked resources from each web page were independently evaluated for information on congenital CMV and were systematically reviewed for presence or absence of information on prevention of congenital CMV infection and accuracy of prevention messages if present.

**Results:** A total of 289 web sites with the term “congenital CMV” were identified. After applying the inclusion criteria, a total of 112 consumer-friendly web sites comprised the final sample. Of those 112 web sites, 44% included prevention information, 17% included partial prevention information and 40% had no prevention information. Finally, a comparative search using a metasearch engine versus a single search engine showed that the latter (the type used by most consumers) resulted in only 15 web sites meeting the criteria with only 8 of those including information on the prevention of congenital CMV.

**Conclusions:** Although information on congenital CMV infection is available on the internet, web sites often do not include information on prevention of congenital CMV. Given that the internet is an important source of health-related information, continuing assessment of web site content for quality and completeness of information is essential. In addition, future analyses should assess whether information on the internet impacts the knowledge and behaviors of women regarding prevention of congenital CMV.
**O-24 A two-year study on Cytomegalovirus infection during pregnancy in a French hospital.**
Christelle Vauloup-Fellous, Olivier Picone, Anne-Gaël Cordier, Isabelle Parent du Cha Telet, Marie-Victoire Senat, René Frydman, Liliane Grangeot-Keros. Service de Microbiologie-Immunologie biologique, Hospital Antoine Béclère, Univ Paris-Sud, Paris, France.

**Background:** Although CMV infection during pregnancy is quite frequent, epidemiological data of this infection, in France, are scarce. To date, vaccines against CMV are not available and treatments of CMV infection during pregnancy have still to be assessed. Therefore, it is important to determine whether modifying potentially risky parental behavior reduces the rate of CMV maternal infections.

**Objectives:** To evaluate the proportion of pregnant women agreeing to cytomegalovirus (CMV) serologic screening. To collect data on CMV infection during pregnancy. To evaluate the efficiency of counseling to prevent maternal CMV infection.

**Method:** CMV screening has been performed in our hospital for years and is performed after informed consent has been signed. The aim of our study was to analyze this epidemiological cohort over two years. The first medical visit was around 12 weeks of gestation (WG). At that time, each pregnant woman was informed on the possible consequences of CMV primary infection and on the lack of scientific data concerning the interest of screening. If patients agreed to CMV screening, serologic testing was then performed after written informed consent. If this first CMV serologic test was negative [absence of CMV-specific IgG and CMV-specific IgM], written (standard script) and oral detailed hygienic information was given to the mother and to the father, and a second test was performed at around 36 WG.

**Results:** Among the 4,287 women followed, 3,792 were either seronegative or with an unknown immune status. 96.7% out of them agreed for screening. 53.2% were initially CMV-specific IgG negative. Primary infection was detected in 9 women (0.52%) between 0 and 12 WG and seroconversion was diagnosed in 5 women between 12 and 36 WG (0.26%).

**Conclusions:** If clear information on CMV infection during pregnancy is given, patients frequently agree to screening. The rate of seroconversion after information observed in this study is low, encouraging counseling.

**Advocacy**

**O-25 Lessons learned from folic acid and how they can be applied to congenital CMV.**
Godfrey Oakley, Jr. MD, MSPM, Research Professor of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA.

In a matter of less than a decade, we went from not knowing the cause of spina bifida to implementing mandatory folic acid fortification of “enriched” grains including flour to prevent this life-altering birth defect. The path to prevention was difficult and lessons learned there may be helpful to the prevention of CMV.

**O-26 How the March of Dimes Has Successfully Influenced Policy to Improve Children’s Health**
Jennifer Howse, March of Dimes Foundation.

Raising over $250 million each year, the March of Dimes operates programs, conducts nonpartisan advocacy, and awards a wide range of grants, all with one aim in mind: to improve children’s health through prevention. Among the chief lessons learned about influencing public policy to improve children’s health are (1) to select the right problem and (2) to create the right strategic networks that can actually attack the problem. For the latter, there must be easily identifiable incentives: for researchers but also for providers and practitioners, for beneficiaries, and for policy makers. Above all, success relies on leadership; leadership that is able to assert a credible public profile to expedite and institutionalize change. One example from the March of Dimes portfolio is its experience, and success at both the federal and state level, with universal newborn screening.
Prenatal Diagnosis, Prognostic Indicators, Correlates of Immunity, and Treatment

O-27 The current state of HCMV prenatal diagnosis
Maria Grazia Revello, Servizio di Virologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

Prenatal diagnosis represents a key option in the management of pregnancy complicated by primary human cytomegalovirus (HCMV) infection in those countries where HCMV testing is performed. Over the years, major factors affecting the reliability of prenatal diagnosis results (including type of specimens, time of gestation at prenatal diagnosis with respect to maternal infection, techniques employed for detection of the virus and/or viral components) have been investigated. As a result, negative and positive predictive values of different techniques are now well defined and, consequently, counseling of pregnant women has greatly improved. Still unaccomplished, however, is the definition of a single, reliable prenatal prognostic marker to be used prospectively on an individual basis. Indeed, only the simultaneous determination of multiple specific and non-specific parameters together with ultrasound examination may help in the identification of infected fetuses at increased risk of congenital disease. Recently, administration of HCMV-specific immunoglobulin has been reported to spectacularly reverse the prognosis in severely affected fetuses (Nigro et al, NEJM 2005). In addition, virological parameters in symptomatic infected fetuses have been shown to possibly improve following valaciclovir administration (Jacquemard et al, BMJ 2007). Should those data be confirmed in controlled studies, voluntary termination of pregnancy would no longer be the only option for mothers of HCMV-infected fetuses. It must be stressed, however, that in the meantime and in the absence of an effective HCMV vaccine, all efforts should be directed to the prevention of maternal primary HCMV infection by means of the identification of seronegative women prior to conception and by providing appropriate information about hygienic measures.

O-28 Findings from CMV hyperimmune globulin treatment trials.
Stuart Adler, Medical College of Virginia, Campus of Virginia Commonwealth University Richmond, VA.

Several options may be available to prevent or treat maternal CMV infection during pregnancy. The first is to enhance public awareness of CMV which would lead to women knowing their risk factors such as serologic status, age of their child, and day care attendance. A second option is for obstetricians to identify high risk women at the first obstetrical visit post-conception. If seronegative, instructing women how to avoid acquiring CMV during pregnancy by hygienic practices should be effective. A third option is for serologic monitoring during pregnancy which would allow the option to terminate the pregnancy or if infected during the first 20 weeks of pregnancy to receive CMV hyperimmune globulin to prevent maternal-to-fetal transmission of CMV. If the fetus is infected the viral load in the amniotic fluid and serial sonograms of the fetus and placenta may predict which fetuses will have a poor outcome. Finally, CMV hyperimmune globulin in an initial study appeared safe and effective for the in utero treatment of babies with poor prognostic ultrasound findings.

O-29 Modeling efficacy of a mAb targeting CMV UL128/130/131: prediction of viral load and mAb affinity.
Jing Yu, Brian R. Stoll, Thomas G. Evans, Teresa Compton, Adam Feire. Novartis Institutes for Biomedical Research, Cambridge, MA.

The UL128/130/131 glycoprotein complex of HCMV was identified as a promising target for the treatment of CMV and the development of a monoclonal antibody (mAb) therapeutic was proposed. We developed a mathematical model to guide the identification of a mAb likely to satisfy the clinical criteria for therapeutic efficacy. The model consists of a standard viral load component combined with a PK/PD component to describe the mAb distribution and binding. The unknown model parameters, such as infection rate and the number of target cells, were determined using in vitro neutralization data and published infection curves for CMV in humans. The model was used to predict the viral load for various mAb affinities, doses, and dosing frequencies. The cases of prophylactic treatment, treatment initiated...
at the viral load detection limit, and treatment for symptomatic patients were simulated. For dosing of 5 mg/kg every 4 weeks, the model predicts that the mAb should have an association rate constant (kon) of 10 \(\text{nM} \cdot \text{d}^{-1}\) and an equilibrium dissociation constant (KD) of 100 pM to ensure viral suppression. For doses of 1 mg/kg every 4 weeks, more stringent binding parameters are required: kon = 100 \(\text{nM} \cdot \text{d}^{-1}\) and KD = 30 pM. For both of these cases, the model predicts an IC50 < 0.1 \(\mu\)g/mL in the in vitro neutralization assay. The development of a model and the use of simulation to predict clinical outcome in the early stages of the project provides a valuable contribution to program strategy and a quantitative basis on which to select lead candidates.

**O-30 Efficacy of Cidofovir analogue HDP-CDV in Guinea Pig Models of Congenital Cytomegalovirus Infection.**

Rhonda D. Cardin, Fernando J. Bravo, Karl Y. Hostetler, David I. Bernstein. Cincinnati Children’s Hospital Medical Center, Cincinnati, OH.

**Background:** Congenital cytomegalovirus (CMV) infection can be life-threatening in newborns and often results in significant deficits in neural development and hearing loss. Available anti-CMV antivirals are effective, but their use is limited due to lack of an effective oral formulation and the significant side effects associated with therapy.

**Objective:** To determine whether the orally active ether lipid ester analogue of cidofovir, hexadecyloxypropyl-CDV (HDP-CDV or CMX001), limits CMV infection in guinea pig models of congenital CMV infection.

**Methods:** Pregnant Hartley guinea pigs were inoculated SQ with guinea pig CMV (GPCMV) during the late second/early third trimester of gestation. HDP-CDV (20 mg/kg) or placebo was administered PO at 1 day and 7 days post infection (dpi) to pregnant animals. HDP-CDV was also administered PO at 1 dpi at 4 mg/kg and continued for 5 days. A second guinea pig model was used to evaluate HDP-CDV treatment on viral replication in newborn guinea pigs following IP inoculation of GPCMV within 48 hours of life. Pups were administered 4 mg/kg HDP-CDV IP or placebo at 1 dpi and continued for 10 days. Pups were sacrificed at 10 dpi and viral load in tissues were evaluated by Real-Time PCR.

**Results:** Pup survival was significantly increased in the drug treated groups, with 94% pup survival in pregnant dams receiving 20 mg/kg (p=0.02) and 100% pup survival in pregnant dams receiving 4 mg/kg for 5 days (p=0.005) compared to placebo (55-60% pup survival). In the first study, viral load in tissues harvested from drug-treated pups was significantly (P<0.05) lower in the spleen (1.7±0.7 Log10 copies/µg DNA) and the liver (1.9±0.8 Log10 copies/µg DNA) compared to the controls (2.5±1 Log10 copies/µg DNA and 2.9±5 for the spleen and liver, respectively), as determined by Real-Time PCR. In the neonatal GPCMV study, the viral load in the spleen, liver, lung and brain of drug treated animals was significantly (P<0.005) lower (1.3-2.8 Log10) when compared to controls.

**Conclusions:** Oral HDP-CDV or CMX001 is well tolerated and effective in limiting CMV infection and may provide an oral alternative to other therapies in improving the outcome of congenital CMV infection.

**O-31 The Frequency of CMV Exposure Among Women Contemplating Additional Pregnancies.**

Beth C. Marshall, Stuart P. Adler. Virginia Commonwealth University Health System, Richmond, VA.

**Background:** Following a primary maternal CMV infection during pregnancy, the transmission rate of CMV from a pregnant woman to her newborn is between 33% and 50%. Children in large group daycare frequently acquire CMV infections from other children, and since many women with a child in day care are planning to bear additional children, these women are at significant risk of a primary infection during pregnancy.

**Objectives:** To determine the frequency of pregnancy and exposure to CMV among mothers contemplating a possible additional pregnancy and with a child less than 2 years of age in group day care.
**Methods:** We performed a prospective observational study which included a demographic questionnaire and serologic and virologic monitoring of mothers and their children in day care. Women planning or contemplating planning pregnancy within 5 years and who had a child less than 24 months of age in day care more than 20 hours a week were invited to participate.

**Results:** Of 60 evaluable women, 62% were seronegative and 20% had a child shedding CMV. Of the 60 women, 23 women or 38% (95% CI = 0.27, 0.51) became pregnant on average 10 months after enrollment. Among seronegative women, 4 of the 7 (11%) who had a child shedding CMV seroconverted prior to becoming pregnant. During pregnancy, eight or 35% (95% CI = 0.19, 0.55) of these pregnant women had a child in day care who shed CMV. None of the seronegative pregnant women (all of whom received hygienic instructions on how to avoid CMV acquisition from their child) seroconverted during pregnancy.

**Conclusions:** These results illustrate the potential magnitude of the public problem associated with exposure to a silent viral infection during pregnancy. Our data, when extrapolated to the U.S. population, estimate that every two years between 31,000 and 168,000 susceptible pregnant women will be exposed to CMV by an infected child.

**0-32 A Proposed Randomized Controlled Trial of Maternal Hyperimmune Globulin to Prevent Fetal Infection.**
Mara J. Dinsmoor, Dwight J. Rouse, Brenna Anderson, George Saade. Evanston Northwestern Healthcare, Evanston, IL.

**Background:** The Maternal Fetal Medicine Unit Network (MFMU), funded by the Eunice Kennedy Shriver NICHD, consists of 14 university centers that collaborate on studies of import to pregnant women and their offspring. (http://www.bsc.gwu.edu/mfmu/)

**Objectives:** To perform a randomized trial of the use of CMV hyperimmune globulin (CMV-HIG) to prevent congenital CMV infection and its sequelae, following maternal primary CMV infection during pregnancy.

**Methods:** The current study protocol involves serologic screening for CMV in gravidas cared for at one of the participating institutions. Screening (CMV IgM and IgG with avidity index) will begin early in pregnancy, and be repeated once, prior to 22 weeks. Gravidas with an initially positive CMV-IgG and low avidity index, and those who seroconvert during the screening period, will be offered enrollment in the trial. Prior to 24 weeks gestation, participants will be randomized to receive monthly infusions of either CMV-HIG (100 Units/kg) or placebo, until delivery or until fetal infection is diagnosed. Amniocenteses will not be routinely performed but are not proscribed. A sample size of 800 subjects will be needed to show a 30% reduction in both congenital infection, using a baseline risk of 33%, and in 2 year CMV sequelae, using a baseline risk of 25%. This study is feasible in the MFMU network, where 3500 gravidas/month < 24 weeks gestation are currently screened for subclinical hypothyroidism. Historically, the network has accomplished high (> 95%) follow-up rates in children. With a 1% primary infection rate, and an estimated 50% consent rate, screening of the necessary 160,000 gravidas can be accomplished in under four years.

**Results:** The primary outcome variable will be congenital CMV infection, the diagnosis of which will require a positive CMV PCR from amniotic fluid or a positive CMV PCR from neonatal urine within the first 2 weeks of life. In a planned secondary analysis, neonates will be followed for 24 months, with annual hearing, neurologic, and ophthalmologic exams. Rates of putative CMV sequelae will be compared between groups.

**Conclusions:** This protocol has received initial approval from the MFMU Steering Committee. Input into study design is currently being solicited.
Vaccines

O-33 Update on CMV gB Vaccine.
Robert Pass, University of Alabama at Birmingham, Birmingham, AL.

Background: A vaccine for CMV is a top public health priority for the U.S. because of the frequency of congenital CMV infection and its importance as a cause of sensory, cognitive and motor disability in children.

Objectives: Review the development of CMV glycoprotein B (gB) vaccine including evidence of efficacy from a recent phase II clinical trial.

Methods: Over the past 14 years multiple phase I and II clinical trials of a recombinant CMV gB vaccine with an oil (squalene) in water adjuvant (MF59) have been conducted and have provided information on safety, optimal immunization dose and schedule as well as immunogenicity of the vaccine. A recent double-blind, placebo-controlled phase II clinical trial of CMV gB vaccine included 441 seronegative young mothers. The primary endpoint was time to CMV infection. Study vaccines (CMV gB, 20 µg or saline placebo) were given on a 0,1 and 6 month schedule by IM injection. Subjects were tested for CMV infection every three months and newborns were tested for congenital CMV infection. Vaccine reactogenicity was assessed using 7 day diary cards for local and systemic reactions and safety was evaluated by careful tabulation of adverse events.

Results: CMV gB vaccine with MF59 is highly immunogenic, inducing antibody to gB, neutralizing antibody and lymphocyte proliferative responses to gB in nearly all vaccine recipients from multiple clinical trials. Comparison of CMV infection rates in the recent phase II efficacy trial using Kaplan-Meier curves showed that CMV gB vaccine recipients were more likely than placebo recipients to remain uninfected over a 42 months interval, P = 0.019, (log rank test). Overall vaccine efficacy was 50%. Three congenital infections (one symptomatic) occurred among 97 live born infants of placebo recipients (3.1%) compared with 1/81(1.2%) among CMV gB recipients. Local reactions occurred more often in CMV gB vaccine recipients than placebo recipients; reactions were generally brief (<48 hours) and well tolerated. Overall adverse events and serious adverse events in mothers and babies born during study occurred with similar frequencies in vaccine and placebo groups.

Conclusions: CMV gB vaccine shows promise for prevention of maternal and congenital CMV infection.

O-34 Induction of Pluripotent Immunity following Immunisation with a Novel Chimeric Vaccine against CMV.
Rajiv Khanna, Jie Zhong, Michael Rist, Leanne Cooper, Corey Smith. Australian Centre for Vaccine Development, Herston, Australia.

Based on the life-time cost to the health care system, the Institute of Medicine has assigned the highest priority for a vaccine to prevent human cytomegalovirus (HCMV) infection in transplant patients and new born babies. In spite of numerous attempts successful licensure of a HCMV vaccine formulation remains elusive. Here we have developed a novel chimeric vaccine strategy based on a replication-deficient adenovirus which encodes the extracellular domain of gB protein and multiple HLA class I & II-restricted CTL epitopes from HCMV as a contiguous polypeptide. Immunisation with this chimeric vaccine consistently generated strong HCMV-specific CD8+ and CD4+ T-cells which co-expressed IFN-γ and TNF-α, while the humoral response induced by this vaccine showed strong virus neutralizing capacity. More importantly, immunization with adenoviral chimeric vaccine also afforded protection against challenge with recombinant vaccinia virus encoding HCMV antigens and this protection was associated with the induction of a pluripotent antigen-specific cellular and antibody response. Furthermore, in vitro stimulation with this adenoviral chimeric vaccine rapidly expanded multiple antigen-specific human CD8+ and CD4+ T-cells from healthy virus carriers. These studies demonstrate that the adenovirus chimeric HCMV vaccine provides an excellent platform for reconstituting protective immunity to prevent HCMV diseases in different clinical settings.
O-35  A Live, Attenuated CMV Vaccine Protects at the Placental-Fetal Interface in the Guinea Pig Model.  
Ryan Buus, K. Yeon Choi, Alistair McGregor, Michael Leviton, Jodi Anderson, Mark Schleiss. Department of Pediatrics, Center for Infectious Diseases and Microbiology Translational Research, University Of Minnesota, Minneapolis, MN.

Congenital human cytomegalovirus (HCMV) infection can lead to long-term neurodevelopmental sequelae, including mental retardation and sensorineural hearing loss. Preconception vaccine strategies can be studied in the guinea pig cytomegalovirus (GPCMV) model. The objectives of this study were: 1) to assess in guinea pigs the protective efficacy against congenital infection of a recombinant live, attenuated vaccine with a targeted deletion of the GPCMV UL83 homolog, GP83; 2) to examine the role of the placenta in vaccine-mediated protection, using in situ hybridization (ISH) with a probe for GP55, the GPCMV glycoprotein B homolog. Outbred Hartley guinea pigs were vaccinated prior to pregnancy with a two-dose series of 5x10^4 pfu of vAM409, a GP83 deletion virus, and examined for DNAemia with real-time PCR. After mating, pregnant animals were challenged with salivary gland-adapted (SG) GPCMV (1x10^6 pfu) in the second trimester, and pregnancy outcomes were compared to unvaccinated controls. Following delivery, placentas were recovered, frozen, cryosectioned, and ISH using a GPCMV GP55 DNA probe was performed. Three sections per placenta were analyzed by a blinded investigator at 20X magnification and recorded as GPCMV positive or negative. Positive cells were counted and divided into groups: low (1-10 positive cells/cm^2), intermediate (11-40 positive cells/cm^2), or high (>40 positive cells/cm^2). The deletion of the GP83 gene significantly attenuated GPCMV, and vAM409 vaccinated animals did not demonstrate evidence of DNAemia. Vaccination significantly reduced maternal DNAemia following SG challenge, and resulted in significantly decreased pup mortality in litters born to vaccinated dams (3/29; 10%), compared to control (35/50; 70%; p<0.001). Recovered placentas from vaccinated and control litters demonstrated placental infection in 6% (2/17) placentas in the vAM409 vaccine group compared to 62% (13/21) in the control group (p<0.001). The number of infected cells in control placentas was variable; 54% low, 23% intermediate, and 23% high. ISH-positive placentas from the vAM409 group demonstrated only low-to-intermediate numbers of GPCMV-positive cells. Preconception immunization with a GP83 deletion vaccine reduced maternal DNAemia and results in protection against congenital GPCMV-associated pup mortality compared to unvaccinated controls. This protection is mediated at the placental level, with vaccination conferring nearly complete protection against placental GPCMV infection.

O-36  Vaccines strategies for therapeutic as well as prophylactic use in preventing congenital CMV.  
Edward Mocarski, Emory University, Atlanta, GA.

Vaccines strategies for therapeutic and prophylactic use in preventing congenital CMV. Edward S. Mocarski, Department of Microbiology and Immunology and Emory Vaccine Center, Emory University, Atlanta GA. Universal vaccine strategies to prevent CMV congenital disease have been focused on CMV-naive populations, with some of these initiatives continue to proceed through testing. Recurrent CMV infection, resulting from either reactivation of latent virus or reinfection during pregnancy, may also result in congenital disease. Although transmission is less frequent in CMV seropositive women, the high percentage of such women in the childbearing years has lead to estimates that this population may be responsible for a greater percentage of CMV congenital infections as well as CMV congenital disease. Therapeutic vaccine strategies to boost either antibody and/or T cell immunity have been described, and should be compared further. Understanding the role of CMV antibody avidity and/or CMV T cell immunity in controlling transplacental transmission will require a greater focus on the CMV seropositive populations. I will discuss the application of available subunit, live attenuated and vector-based vaccines in therapeutic vaccination, using studies in mice as a pre-clinical model. I will also discuss the evidence to date on strategies likely to be useful in boosting a natural memory immune response to CMV.
Newborn Screening

0-37 Current Screening Policy in the United States
R. Rodney Howell, Professor of Pediatrics, Miller School of Medicine, University of Miami, Miami, FL.

Routine newborn screening (NBS) has been carried out as a state-run public health program in all of the 50 states since the 1970s. Beginning with phenylketonuria for which screening has long been universal, there has been widespread variation from state to state in adding other diseases to the NBS panel. In 2001, HRSA/HHS contracted with the American College of Medical Genetics to develop a recommended newborn screening panel. This widely representative working group concluded that all infants should be screened for 29 conditions designated the “core panel”. At the same time, the Congressionally-mandated Secretary’s Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) unanimously supported the ACMG recommendation. Currently the vast majority of infants born in the United States are tested for this “core panel”. The ACHDNC has since developed a systematic approach for nominating and evaluating conditions to be considered in the future for NBS. This approach includes a detailed, transparent evidence review system that will guide future recommendations. Since over 4,100,000 infants were born in the US in 2006, newborn screening is by far the most frequently performed genetic testing in the United States. The September 19, 2008 MMWR estimated that the adoption of the ACMG panel in all 50 states (nearly accomplished) would increase the number of children identified with serious, treatable conditions from 4,370 to 6,439, a 32% increase. NICHD has recently funded a program that will focus a research agenda on the long-term follow-up of children identified during newborn screening. A comparison of the US approach to newborn screening to other developed countries shows great variation among these countries. In the UK only a few conditions are routinely screened for in the newborn period while Austria and others have extensive screening panels similar to that recommended by the US Secretary’s Advisory Panel.

0-38 Issues Related to Congenital CMV Newborn Screening.  
Scott Grosse, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA.

Newborn screening can be done as a clinical service or as a public health program. For a public health screening test, key issues are 1) the availability of an accurate and relatively inexpensive test, 2) demonstration of benefit to affected individuals, and 3) lack of harm. For congenital CMV, challenges include 1) developing and validating high-throughput dried blood spot assays that use an acceptably small quantity of blood; 2) assessing the predictive power of tests to identify children at risk of sequelae, notably late-onset or progressive hearing loss; and 3) assessing the potential benefits and harms of identifying asymptomatic children with congenital CMV, 12%-15% of whom will develop sequelae. The opportunity to intervene early in case of hearing loss or developmental delay must be balanced against parental anxiety. If there were safe treatments that could prevent the onset of hearing loss, screening for congenital CMV would be more compelling.

0-39 DECIBEL-study: Congenital Cytomegalovirus infection and newborn hearing screening strategies.  

Background: Congenital cytomegalovirus infection is the most important non-genetic cause of permanent childhood hearing impairment (PCHI) and may cause developmental delay. Hearing loss can be apparent at birth, delayed in onset or progressive. The DECIBEL-study is a nationwide retrospective, observational, pseudo-randomized study in children with PCHI in the Netherlands. In this study the region and date of birth determine the type of hearing screening strategy since neonatal hearing screening (within 2 weeks of birth) gradually replaced the distraction hearing screening (at 9 months) between 2002 and 2006. General development and congenital CMV-DNA detection are primary outcome measures in comparing children in both hearing screening strategies.
Objectives: 1) Determine the prevalence of congenital CMV infection in 3-5 year old children with PCHI in the Netherlands, 2) quantify the effect of the screening strategy on the age at PCHI detection in children with congenital CMV, 3) describe the developmental outcome of these children.

Methods: Children born in the Netherlands between 1-1-2003 and 31-12-2005 with PCHI (Definition: hearing deficit of ≥40dB in the better ear) registered at Audiological Centres are eligible for participation in the DECIBEL-study providing they have been offered a Dutch hearing screening strategy in the first year of life. Assessments: CMV DNA detection on neonatally acquired dried blood samples. The Child Development Inventory-NL to investigate general development and additional developmental- and audiological parameters from medical records.

Results: 482 children (born 1-1-2003 to 31-12-2005) were registered with PCHI at Audiological Centres in the Netherlands. Preliminary results of CMV-DNA testing in children in the DECIBEL-study show approximately 10-20% CMV-DNA positivity. Of the 3-5 year olds with PCHI and congenital CMV infection, more children had passed the neonatal hearing screening strategy than children in the (later) distraction screening hearing strategy. Results of the CDI-NL show a small overall delay in children with PCHI and congenital CMV.

Conclusions: Early hearing screening strategies produce some negative screening results in children with congenital CMV infection, presumably because of progressive and delayed onset hearing loss. Developmental delay often accompanies PCHI in these children.

O-40 Evaluation of DNA extraction methods using dried blood spots for diagnosing congenital CMV infection.

Background: Cytomegalovirus (CMV) is the most common cause of congenital infection worldwide and occurs when CMV is transmitted from mother to fetus. The most frequently encountered symptom of congenital CMV infection is sensorineural hearing loss (SNHL). The only reliable method to diagnose congenital CMV in older children is CMV detection in dried blood spots (DBS) sampled within one week after birth. Since the first publication on the usage of DBS for diagnosing congenital CMV infection, several DNA extraction methods for DBS followed by CMV PCR have become available. However, a recent quality assessment programme by Quality Control for Molecular Diagnostics (QCMD) studying CMV DNA detection in DBS among European and South African laboratories showed that the assays as employed had a worrisome lack of sensitivity as well as specificity.

Objectives: To compare DNA extraction methods for DBS for CMV DNA detection. Sensitivity, specificity and suitability of the methods for high-throughput usage were assessed.

Methods: Guthrie cards were spotted with CMV-positive whole blood from transplant recipients with a broad range of CMV plasma loads. DNA was extracted from DBS using a panel of six non-commercial and commercial DNA extraction methods, followed by CMV real-time PCR amplification. Samples were tested in triplicate with negative control punches between each sample.

Results: DBS with high CMV loads (plasma loads ≥ 4 log10 c/ml) were positive in all triplicates of all DNA extraction methods tested. However, using DBS with lower CMV loads, the ability to detect CMV DNA decreased markedly, depending on the method used. Furthermore, CMV loads extracted from the DBS varied widely. Specificity of the methods tested was high. Some extraction methods appeared suitable for medium-throughput applications, only few methods appeared suitable for automated applications using a 96-wells format.

Conclusions: The differences in sensitivity and capacity of the DNA extraction methods tested limit the possible applications. Both highly sensitive and high-throughput methods are required when considering newborn screening for congenital CMV infection.
PCR Detection of Congenital CMV Infection in Minnesota Infants Failing Newborn Hearing Screening.

K. Yeon Choi, Lisa A. Schimmenti, Mark McCann, Mark R. Schleiss. University of Minnesota, Department of Pediatrics, Center for Infectious Diseases and Microbiology Translational Research, Minneapolis, MN.

**Background:** CMV is a major cause of congenital infection in newborns leading to disabilities such as sensorineural hearing loss (SNHL) and developmental delay. Functional newborn hearing screening (NHS) does not provide information about potential etiologies of SNHL. Rapid diagnosis of congenital CMV infection in newborn who fail the NHS could provide the opportunity for more timely intervention, such as antiviral therapy.

**Objective:** These studies sought to examine newborn blood spots (NBS) from an anonymized group of infants who had failed NHS for the presence of CMV DNA by real time PCR assay.

**Methods:** NBS were obtained from infants who had failed NHS from the Minnesota Department of Health. Real time PCR assay was performed on the Lightcycler® for detection and quantification. A 240 bp fragment of the UL54 (DNA polymerase gene) was amplified with specific primers and FRET probes for CMV UL54.

**Results:** Of 479 children referred for further evaluation because of a failed NHS, there were 13 CMV positive blood spots (2.7%) with a mean viral load of 1.89 x 10^3 genomes/microgram of total DNA (SD, 1704). This compared to only 2/479 positive results from a control group of infants who passed the NHS (0.4%; p=0.003, Fisher’s exact test).

**Conclusion:** Real time PCR is a sensitive technique for identification of CMV DNA from NBS. In Minnesota, approximately 3% of newborns who failed NHS have congenital CMV infection. NBS screening may be a useful and rapid adjunct to functional NHS and enable more rapid etiologic diagnosis of SNHL in newborns. Of interest, 1/13 CMV positive newborn blood spot from the referred group also showed a GJB2 mutation in both alleles.
Poster Abstracts
Epidemiology

P-01 Congenital Cytomegalovirus infection surveillance in a cohort of newborns from Apulia Region (7 yrs). Calvario Agata, Nicola Laforcia, Maria Scarasciulli, Lucrezia de Cosmo, Anna Bozzi, Francesco Schettini. Microbiology and Virology Unit, Laboratory of Molecular and Cellular Virology, Policlinico of Bari, Bari, Italy.

Background: The risk of congenital Cytomegalovirus (CMV) infection is related to maternal antibodies status. In absence of systematic studies on CMV seroprevalence in fertile females in Apulia region, the Neonatology Unit in collaboration with the Virology Laboratory (Bari, Policlinico Hospital) started a surveillance program of congenital CMV infection.

Methods: From 2000 to 2007, 170 newborns admitted in the Neonatology and NICU of Bari University (1900 births/year) have been included in this study, according to the following inclusion criteria: undefined maternal CMV serostatus or possible seroconversion/reactivation in pregnancy; presence of signs suggestive of congenital CMV infection. Cultural and molecular test on urine within the second week of life were carried out to assess congenital CMV infection. In case of positivity, blood samples and/or neonatal Guthrie Cards (DBS) were also tested by Real Time PCR (Q-CMV Real Time; Nanogen Advanced Diagnostics Srl). All newborns CMV infected were included in a follow up program until to 6 years of age.

Results: Seventeen newborns (12 F;5 M) resulted congenital CMV infected. According to maternal serologic status, 7 were born from mothers with seroconversion in pregnancy, ascertainment (AS) in 5 cases and presumed (PS) in 2 cases; 7 from mothers with undefined serology (US) and 3 from mother with possible reactivation (PR). 10 were CMV symptomatic; of these, 5 developed neurologic sequelae (2 cases from US mothers, 1 PS and 2 AS). 3 had normal neurosensorial development (all AS) and 2 were missed to follow up (1 PS, 1 US). 7 congenital infected children were asymptomatic at birth; of these, 2 developed neurosensorial impairment (1PS, 1PR). 2 had normal follow up, the remaining 3 cases were missed to follow up (2 ND, 1PR). None of the newborns received antiviral treatment.

Conclusions: In a recent report we have found that in Apulia region the seroprevalence among fertile women was 74.5% whereas the amount of undefined maternal serological patterns with risk of CMV transmission was 22%. In our study in 42.2% of CMV congenital infected babies, maternal CMV serostatus was incomplete or not ascertained; 36.3% of this group showed cognitive/motor deficit or exitus related to CMV infection.

P-02 Cytomegalovirus Seroprevalence in the U.S. from 1999 to 2004.
Michael J. Cannon, Sheri Lewis Bate, Sheila C. Dollard, Deanna Kruszon-Moran. National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Background: Congenital cytomegalovirus (CMV) is the most common congenital infection in the U.S., resulting in permanent disabilities such as mental retardation, vision loss, and hearing impairment for more than 5,500 children per year. Seroprevalence studies of CMV-specific-IgG provide important data to monitor trends in CMV prevalence, identify risk groups, and assess the potential burden of CMV infection.

Objectives: The purpose of this study is to provide a current and robust estimate of the seroprevalence of CMV in the U.S. by using National Health and Nutrition Examination Survey (NHANES) seroprevalence data from 1999-2004.

Methods: 15,310 stored serum specimens from individuals 6 to 49 years who participated in NHANES 1999-2004 were tested for CMV-specific IgG antibody. To account for the complex survey design of the NHANES dataset, all prevalence estimates were weighted to represent the U.S. population. CMV seroprevalences by demographic and sexual risk factors were assessed. Logistic models were created for each race/ethnic (non-Hispanic White, non-Hispanic Black, and Mexican-American) and gender subgroup due to interactions between age and gender.

Results: The overall age-adjusted seroprevalence of CMV infection for individuals 6 to 49 years was 50.4% (95% Confidence Interval [CI], 48.0%-52.7%). Seroprevalence varied by gender (55.5% [95% CI, 53.3%-57.7%] for females and 45.2% [95% CI, 42.4%-48.0%] for males) and race-ethnicity (39.5% [95% CI, 36.9%-42.2%] for non-Hispanic Whites, 70.6% [95% CI, 68.5%-72.8%] for non-Hispanic Blacks, and 76.9% [95% CI, 74.1%-79.6%] for Mexican-Americans). Seroprevalence steadily increased with age (from 37.5% [95% CI, 34.2-40.8%] for 6-11 years to 58.0% [95% CI, 54.8%-61.1%] for 40-49 years). CMV seropositivity was strongly associated with low socioeconomic status (SES) (ORs ranged from 1.9 to 3.9 for low vs. high SES [poverty index < 1.3 versus > 3.5]) and birthplace outside the U.S. (ORs from 2.8 to 6.3 for birth outside vs. within the U.S.) in all race/gender specific logistic models also adjusted for age and health insurance status (p<.05). For adult women (20-49 years) of each race/ethnic group, education level less than high school was significantly related to CMV seroprevalence (ORs ranged from 2.7 to 3.1 for < H.S. vs. > H.S., adjusting for age, sex, birthplace, insurance, number of lifetime sex partners, and age of sex initiation, p<.05). Number of lifetime sex partners was a significant risk factor for CMV seroprevalence for adult non-Hispanic Whites (OR 1.5
for males and 2.1 for females for >24 partners vs. < 25 partners, p<0.05), and age of sex initiation was a significant risk factor for non-Hispanic Blacks (OR 1.8 for males and 7.8 for females for <14 years vs. ≥15 years, p<0.05), after adjusting for all previously mentioned covariates.

Conclusions: Racial, socioeconomic, and other disparities in CMV seroprevalence continue to persist. Given the association of CMV infection with numerous demographic and sexual risk factors, intervention strategies may need to target specific risk groups.

P-03 Methodologic Concerns in Case-Control Study of Congenital CMV and Infant Prematurity Florida, 2008.

Background: Cytomegalovirus (CMV) is the most common congenital infection worldwide, affecting 0.5%-1.0% of newborns. Case reports indicate congenital CMV might contribute to preterm birth. CMV polymerase chain reaction (PCR) testing of dried blood spots (DBS), collected on all newborns, might prove useful in identifying associations between congenital CMV and preterm birth. However, obtaining DBS and linking them to useful data pose multiple challenges.

Objectives: To plan a case-control study of the association between congenital CMV infection and preterm delivery, including (1) design a method to link birth certificates and newborn screening DBS laboratory records, resulting in a deidentified data set; (2) obtain a high linkage rate (>95%); (3) use the linked data set to locate 1800 stored DBS in the state public health laboratory; and (4) assess if quality of located DBS is adequate to perform PCR testing to identify congenital CMV infection (>98%).

Methods: Database work was performed by the Florida Department of Health. Duplicate DBS records were eliminated, and DBS and birth records were linked by computer on the basis of patient identifiers, including birth facility, date of birth, and infant's name. Linking criteria were subsequently modified 14 times to achieve a high linkage rate. Final links were performed manually. A stratified random sample of 2,000 records was selected to include a predetermined proportion of full-term, late preterm, and early preterm infants. All identifiers except the laboratory accession number were stripped from the linked records before the DBS were selected from storage. The final data set contained clinical and demographic variables and a study number but no personal or newborn screening laboratory identifiers.

Results: Ninety-eight percent of DBS records were successfully linked with birth records (17,970/18,376). All specimens sought were located, and >99% (1,794/1,800) had sufficient material for CMV testing.

Conclusions: The methodology provides adequate data linking and collection of DBS for congenital CMV infection testing. Linking rates and specimen collection and quality exceeded minimum study goals. We collected specimens without personal identifiers and constructed a deidentified analysis data set. These methods might be useful for future CMV and other congenital disease investigations.

P-04 Congenital CMV Disease (C-CMV-D) Registry 1990-2007: Targets for Treatment and Prevention Revealed.
Gail Demmler-Harrison, Carol Griesser RN, Daniel Noyola MD, Malorie Snider, and Congenital CMV Registry Participants. Baylor College of Medicine, Houston, TX.

Background: C-CMV-D causes neonatal morbidity and mortality and neuro-sensory-developmental disabilities. In collaboration with the CDC and CMV investigators, a CMV Registry was established in 1990 in Houston, to monitor C-CMV-D trends over time, identify targets for prevention, raise CMV awareness, and facilitate collaborations.

Objectives: Define demographics of mothers and characteristics of newborns with C-CMV-D, compare data with national statistics, and describe trends over time, to determine emerging or priority targets for intervention and prevention.

Methods: Voluntary one page case reporting system, submitted by physicians, with data verified and transferred to electronic database (SPSS) for descriptive statistics and comparisons between case groups and with national norms.

Results: 782 cases from all 4 U.S. regions reported over 17 years. Means were maternal age 22.6 yr, GA 36.2 wks, BW 2254 g, HC 30.5 cm. Most common newborn signs included petechiae/thrombocytopenia (57%), hepatosplenomegaly (49%), SGA (47%). 70% had CNS involvement (hearing loss 43%, intracranial Ca+ 43%, and/or microcephaly 39%). Neonatal death was reported in 6.8%. Cases > 32 wk GA were more likely than <32 wk GA to have petechiae (60% vs 38%), splenomegaly (47% v 37%), intracranial Ca+ (47% v 23%), ALT > 100 U/L (33% v 13%); whereas pneumonitis (19% v 9%), thrombocytopenia (65% v 23%), and neonatal death (19% v 5%) were more likely in <32 wk GA.
C-CMV-D cases were more likely than national statistics (CDC 2004) to be preterm (42% v 12%), LBW (63% v 8%) and have mothers < 18 yr (27% v 6%) of Black race (33% v 14%). Bimodal distribution of maternal demographics revealed 72% < 25 yr group (peak 19 yr), White to Black ratio 1.3:1, more likely Medicaid (69%), unmarried (68%), primagravid (59%); whereas mothers > 25 yr (28% of cases) had White to Black ratio 5.8:1, were more likely to have private insurance (61%), be married (82%) and multigravid (84%). Analysis of 17 yr trends did not show changes in maternal or neonatal characteristics, except for possible increase in White/Hispanic mothers, consistent with national statistics. Antiviral treatment increased over time (2% in 1990-1995 v 44% in 2002-2007), but neonatal death rate remained constant (6% to 8%).

Conclusions: C-CMV-D is associated with LBW, preterm birth, and neonatal death, and is an important target for awareness, treatment and prevention. Presentation of C-CMV-D in < 32 wk GA differs from > 32 wk GA newborn, suggesting acute viral sepsis syndrome amenable to antiviral therapy. Maternal targets for prevention include two high risk groups (unmarried, primiparous, minority teenage mothers and married, multiparous mothers > 25 yrs of white race.

P-05 Cytomegalovirus infection in day care centers of Sao Paulo, Brazil. A descriptive study.
Daniela Forlin, Fernando Colugnati, José Augusto A.C. Taddei. UNIFESP, Federal University of São Paulo, São Paulo, Brazil.

Background: Cytomegalovirus (CMV) infection is a frequent cause of congenital and perinatal infection and a major source of disability. Few studies described CMV immunological status for low income infant population in developing countries. Moreover, epidemiology of infection is not well established in Brazil.

Objectives: Determine the IgM seroprevalence of CMV and describe socio-economic, demographic and epidemiological characteristics of infants and toddlers attending public day care centers of São Paulo.

Methods: Brazilian children (n=252), ages 9-38 months, both genders, attending eight public day care centers in a low socio-economic district in São Paulo were evaluated. A DBS card was used for blood sample taken from the child’s finger stick. CMV IgM seroprevalence was measured by the Neo Map IgM Kit Torch Multiplex System. The database was input and analyzed by EpiInfo 2000 and Stata 10 computer systems. The socio-economic, hygiene and demographic status, pregnancy, birth and current health factors, such as length of breastfeeding and nutrition status were obtained from a questionnaire answered by the mother.

Results: IgM seropositivity was 3.2%. The mean age of children was 24 months, genders were well distributed (51.6% male and 48.4 female), and 97.2% of subjects were born in the city of São Paulo. Mothers were the primary caregivers, their median age was 28 and 41.8% of them were unskilled workers, the mean level of their education was 8 years. Mean daily income per capita was 3.9 dollars. Of the sample children, 39.5% were the result of an undesired pregnancy, 13.1% weighed under than 2.5 kg at birth and 23% had siblings under the age of five. One in ten had been hospitalized over the last six months. Most (98.8%) had been fully vaccinated, 49.2% use pacifiers and 86.5% are bottle fed. Median period of breastfeeding was 90 days. 21.8% were anemic (Hb <11.0 g/dL) and 10.1% were malnourished (WAZ score < -1.5).

Conclusions: Prevalence of CMV IgM seropositivity shown in this study was low, although the described population is a very susceptible group for CMV acquisition and transmission.

P-06 Day-care occupational risk of CMV infection is highest during the first two years of employment.

Background: Day care personnel have multiple risk factors for CMV infection related to their work in the day care setting, as well as personal risk factors.

Objectives: We established the occupational risk of CMV infection in Dutch female day-care personnel in relation to working seniority with adjustment for personal risk factors.

Methods: All participants received a questionnaire concerning demographic data, family setting, occupational years and day-care facility characteristics. CMV IgG seroprevalence was determined by standard methods (AxSym, Abbott) in serum samples of 319 females, recruited form 56 regional day-care facilities. Seroprevalence was also determined in a control group of 158 female students.

Results: Logistic regression analysis indicated an increased risk for cytomegalovirus in female day care personnel with an overall adjusted Odds ratio of 2.47 (95% CI: 1.46-4.18). This increased risk was most prominent in the personnel subgroup of 1-2 years working seniority with an Odds ratio of 3.64 (95% CI: 1.46-9.08). In the personnel
subgroups with 3-4, 5-9 and >9 years working seniority, Odds ratios declined to 2.09, 2.07 and 1.13 respectively and were no longer statistically significant. Similar Odds ratios in literature may become higher and more significant if reanalyzed and focused on subgroups of day-care personnel and other health care workers with limited working experience.

Conclusions: The increased risk of a CMV primary infection in day care personnel is related to working seniority and is most prominent in a high risk group of personnel with only 1-2 years working experience. The identification of high risk groups may be helpful, but also a challenge for current and future guidelines for prevention of primary and/or congenital cytomegalovirus in (pregnant) day care personnel and health care workers.

P-07 CMV Seroconversion and Shedding in CVL Specimens among Women with or at Risk for HIV Infection.
Laura Graham, Michael J. Cannon, Minal Amin, Lytt Gardner, Robert S. Klein, Kenneth Mayer, Anne Rompalo, Jack Sobel, Sheila C. Dollard. National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Background: Understanding factors associated with CMV shedding has important implications in virus transmission but there is limited research on longitudinal shedding patterns in high-risk populations.

Objectives: The purpose of this study was to estimate the rate of seroconversion among CMV-seronegative women as well as rates of and risk factors for cervicovaginal lavage (CVL) shedding over time among CMV-seropositive women.

Methods: We studied a subset of women who participated in the HIV Epidemiology Research Study (HERS). 369 women were followed every 6 months for a median of 5.4 years, contributing a total of 3,333 patient visits. We measured CMV seropositivity and CMV DNA shedding in CVL at each visit and assessed risk factors using generalized estimating equations.

Results: The incidence of CMV seroconversion was 2.6/100 person-years (1.2-5.0 / 100 py). Among seropositive women, 21 (6.1%) were shedding CMV DNA in their CVL at baseline and 99 (33.1%) shed CMV at least once during the study. At visits where shedding occurred, the median CMV viral load was 3.3x102 copies/ml (range=7.1x101 - 7.4x105). There was a strong positive correlation between CMV viral load on consecutive visits (r=0.62, p=0.0001) and the probability of shedding at the next visit was significantly higher for individuals shedding at the current visit than for those not shedding at the current visit (41% and 5%, respectively, p<0.0001). In multivariate models, women who shed CMV were more likely to be HIV positive (OR=5.7, p<0.0001) and younger in age (p=0.0007) than those that did not shed. Among HIV positive women, younger age (p=0.01), increasing HIV viral load (p<0.0001), decreasing CD4 cell count (p=0.008), and bacterial vaginosis (OR=1.7, p=0.02) were associated with shedding CMV.

Conclusions: Reactivation or reinfection is common in populations with or at risk for HIV infection. We found that nearly 1/3 of the women were shedding CMV in CVL during a 6-year period, although due to the selection of participants this may somewhat overestimate shedding in the overall HERS population. CMV shedding in CVL is often sporadic but it is correlated with previous incidents of shedding. Immunosuppression and other genital-tract infections may promote CMV shedding.

P-08 Prevalence and quantification of cytomegalovirus viral shedding in toddler’s (1-6 Yr old) saliva.
Jérôme Grosjean, Laurane Trapes, Benoît Marin, Sébastien Hantz, Catherine Mengelle, Philippe Brosset, François Denis, Sophie Alain. Faculté de Médecine de Limoges de Bactériologie-Virologie, Centre Hospitalier Universitaire de Limoges, France.

Background: Cytomegalovirus infection is the first cause of viral congenital infection, and a vaccine priority since 2000. The women most exposed to in utero transmission are seronegatives women (in France 55 % of pregnancies), particularly those in contact with young children in day care centers (DCC). In this context, vaccination is an important objective and epidemiology of CMV in this reservoir is essential.

Objectives: To assess the feasibility of CMV quantification in toddler’s saliva and study CMV prevalence from this population sample.

Methods: A feasibility study was carried out in 625 children of less than 6 years (369 children from the emergency department of Limoges teaching hospital and 256 children from 6 day care centers). Salivary sampling was chosen for its noninvasive character. We adapted a kit from DNAgenotek using sponge swabs with culture or DNA conservation medium. Demographic, medical and practice of life informations were obtained for each participating child and entered into a standardized collection report form. For each toddler a small clinical examination (adenopathy, fever ) was added.
**Results:** Detection by PCR was positive in 80 children (21.7%) and negative in 289 (78.3%) at the emergency unit. In DCCs, global prevalence was 51.9% but prevalence differed notably among DCCs (0% to 74.7%) showing a center-related effect (p<0.0001). High prevalence was found in big DCCs. Average shedding was about 5.6 Log copy/mL at the emergency unit and in DCCs, but with a bimodal distribution in emergency and unimodal in DCC. At the emergency low children's age (p=0.019), other infectious illness (p=0.03) and presence of other children under 3 at home (p=0.04) were correlated with high level of viral shedding. In DCCs center-related effect was the only significant factor of viral shedding, and high level shedding was observed between 3 and 9 months of cumulated attendance in the DCC.

**Conclusion:** We show that saliva sampling will facilitate further epidemiological study of CMV shedding at the source of congenital infection. High viral load is clearly related with low age in general population but not in DCCs.

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**P-09**  
**Congenital Cytomegalovirus Infection (CCI) in Israel in the Era of Unofficial Prenatal Screening.**  
Nina Hirsch, Smadar Eventov-Friedman, Dov Brown, Niveen Saleh, Zion Hagay, Dana G. Wolf. Kaplan University Medical Center, Jerusalem, Israel.

**Background:** CCI is a considerable public health problem, yet no routine screening programs in pregnant women have been formally established. While prenatal screening holds promise as a preventive measure, its impact on the prevalence and outcome of CCI has not been evaluated.

**Objectives:** To determine the prevalence of CCI in newborns of mothers subjected to a high rate of unofficial prenatal screening, and to relate the occurrence and outcome of CCI to the type of maternal infection.

**Methods:** Consented live-born infants born at the Kaplan University Medical Center over a 1-year period were screened prospectively for the presence of CCI by real time PCR in cord blood, urine and saliva samples. Maternal infection was categorized based on serological testing at labor and retrospectively retrieved serological data. The presence of congenital cytomegalovirus disease was determined by hearing and developmental evaluation over a 1-year follow-up period.

**Results:** 3103 newborns, representing 55.7% of live-born infants in the hospital during the study period, were tested. The rate of prenatal screening among consenting mothers was 85.5%. CCI was identified in 20/3103 infants (0.64% prevalence; 95% confidence interval 0.367-0.921). The rate of prenatal screening among consenting mothers of infants with CCI was 94.4%. Of the 20 congenitally infected newborns, 8 (40%) were born to mothers with primary third trimester infection, 3 (15%) - primary first or second trimester infection, 8 (40%) - non-primary infection, and 1 uncategorized. Cord blood viral load ranged from 2.3 to 5.8 log copies/mL (median 3.9). Two newborns of third trimester infection demonstrated only transient laboratory abnormalities at birth. Hearing deficiency was detected at 6m in one child of second trimester infection.

**Conclusions:** A predominance of late and non primary maternal infection was revealed among congenitally infected infants in a population with a high rate of prenatal screening. This observed distribution could reflect the changing epidemiology of CCI in the era of prenatal screening with a possible reduction in disease prevalence.

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**P-10**  
**Potential Economic Benefits of Preventing Congenital CMV Infections in the United States.**  
Scott Grosse. Centers for Disease Control and Prevention, Atlanta, GA.

**Background:** The Institute of Medicine in 2000 prepared preliminary estimates of the potential economic benefit of a hypothetical vaccine to prevent congenital CMV infections, but those calculations were based on outdated estimates of the prevalence of congenital CMV, the frequency of congenital CMV sequelae, and the costs associated with disabling sequelae.

**Objectives:** The purpose of this study is to present updated estimates of the economic costs associated with congenital CMV infection based on recently published research on the prevalence and sequelae of congenital CMV infections and costs associated with disabling sequelae and infant mortality in the United States.

**Methods:** Recently published U.S. estimates of congenital CMV prevalence (0.7%), infant mortality (0.5%) and neurological sequelae, including permanent, bilateral hearing loss of 40 dB or greater without other sequelae (2-4%) and intellectual disability (4-7%) are combined with estimates of the lifetime direct and indirect (productivity) costs in the United States adjusted for inflation to 2005 US dollars associated with infant death ($1.2 million), intellectual disability ($1.2 million), and bilateral moderate to profound pre-lingual hearing loss ($0.5 million) discounted to present values using a 3% discount rate and assuming 1% per year future growth in productivity and incomes.

**Results:** Each case of congenital CMV infection prevented is conservatively calculated to result in $63,000 to $108,000 in averted costs associated with infant mortality, bilateral hearing loss, or intellectual disability. The economic cost
associated with such outcomes in each year’s birth cohort with congenital CMV infections is estimated to be $1.9 to $3.2 billion dollars.

Conclusions: The potential economic benefits from the primary prevention of congenital CMV infections are enormous and help to justify the allocation of resources to the development of vaccines targeted to congenital CMV as well as to the testing of health promotion strategies to reduce infections among pregnant women. The estimates presented here are conservative because they do not include sequelae for which either cost evidence (mild or unilateral hearing loss) or quantitative epidemiologic evidence (visual impairment) is lacking.

P-11 Retrospective Surveillance of Congenital CMV Disease at School for the Deaf by Using Umbilical Cord.
Masako Moriuchi, Masato Tagawa, Hideo Tanaka, Hiroyuki Moriuchi. Nagasaki University Graduate School of Biomedical Science, Nagasaki, Japan.

Background: Congenital cytomegalovirus (CMV) infection is the leading cause of sensorineural hearing loss (SNHL) in developed countries. Since SNHL is often recognized beyond neonatal period, dried blood spots on Guthrie cards have been used to make retrospective diagnosis of congenital CMV infection. In Japan, however, Guthrie cards are discarded within a year; therefore, retrospective diagnosis had been extremely difficult beyond infantile period.

Objective: To demonstrate an impact of congenital CMV infection at a setting of a school for the deaf by performing retrospective surveillance.

Methods: Retrospective diagnosis of congenital CMV infection was made by detecting viral DNA with real-time PCR from preserved dried umbilical cords of students or graduates of the Nagasaki-Prefectural School for the Deaf where most children in the prefecture who need special educational aids solely for hearing impairment are to attend.

Results: Three (12%) among 26 subjects tested were diagnosed congenital CMV infection. None of them had a family history of SNHL and drew particular medical attention at birth; however, modest intrauterine growth retardation was pointed out in two (67%) of them, and a mother of one patient had a history of aseptic meningitis during her pregnancy. SNHL was asymmetric in two (67%), at delayed onset in one (33%), and progressive in two (67%) of the three. In contrast, among 23 students who were negative for CMV, 9 (39%) had a family history of SNHL, two (9%) had intrauterine growth retardation, one (4%) had asymmetric HL, and none had delayed-onset or progressive HL.

Conclusions: The use of preserved umbilical cord allowed us to perform retrospective surveillance of congenital CMV infection, and demonstrated that the impact of congenital CMV infection on SNHL appeared significant among those with SNHL but no other neurological problems.

P-12 Prospective Surveillance of Congenital Hydrocephalus in Nagasaki, Japan: Impact of CMV Infection.
Hiroyuki Moriuchi, Masanori Egashira, Masako Moriuchi, Kazutaka Ohsawa, Hiroshi Sato, Hideaki Masuzaki. Nagasaki University Graduate School of Biomedical Science, Nagasaki, Japan.

Background: Congenital hydrocephalus has been detected in approximately 5 to 10 per 10,000 live births. It is not a single entity but involves a variety of etiologies, including genetic disorders and congenital infections such as CMV, other TORCH complex agents and lymphocytic choriomeningitis virus (LCMV). However, very few prospective studies have investigated a role of congenital infections.

Design and Methods: A prospective clinicoepidemiological study to demonstrate an etiological role of intrauterine infections in congenital hydrocephalus in Nagasaki, Japan. Between April in 2003 and March in 2008, almost all pregnant women in Nagasaki Prefecture underwent fetal ultrasound studies. All pregnant women suspected of fetal hydrocephalus were referred to one of the three secondary/tertiary hospitals for further investigation and perinatal care. All infants with hydrocephalus or dilated ventricles were registered and clinicoepidemiological data were collected. With informed consent, peripheral blood was drawn from mothers and infants to perform serological tests for TORCH complex pathogens (T. gondii, rubella virus, CMV, herpes simplex virus (HSV), and syphilis) as well as LCMV. Furthermore, real-time PCR was performed for CMV DNA.

Results: A total of 24 infants were identified to have developed congenital hydrocephalus during the 5-year study period, and the incidence was estimated to be 4.9 per 10,000 live births. Among them, two mothers were positive for HSV IgM, but their infants had no evidence of HSV infection. None of them had positive T. gondii IgM, rubella IgM, CMV IgM, RPR or LCMV IgG. Two (8.3%) were positive for CMV DNA by real-time PCR, despite of negative CMV IgM and a lack of other clinical symptoms and signs suggestive of congenital CMV infection.

Discussion: Congenital CMV infection accounted for 8.3% of congenital hydrocephalus in Nagasaki, Japan, and congenital hydrocephalus can be the only clinical presentation in infants with congenital CMV infection.
**P-13**  **CMV gN genotypes among infants with CMV infection at a tertiary hospital in India.**
Sunil Kumar Pati, A. Rajkumar Patro, Ashok Kumar Deorari, Shobha Broor, Lalit Dar. Department of Microbiology, AIIMS, New Dehli, India.

**Background:** Human cytomegalovirus (HCMV) is frequent cause of congenital viral infection. Congenital and perinatal CMV infection rates are higher in populations of developing countries with high CMV seroprevalence. The distribution and diversity of circulating CMV strains in infants with CMV infection in India is not known.

**Objectives:** To examine the CMV strain diversity in infants with CMV infection at a tertiary care hospital, Delhi, India by determining the glycoprotein N (gN) genotypes.

**Methods:** Infants <6 months of age, suspected to have CMV infection, and referred by Department of Pediatrics, All India Institute of Medical Sciences, Delhi between January 2004 and August 2007 were studied. The inclusion criteria are: neonatal hepatitis, small for gestation age, microcephaly, petechiae, purpura, chorioretinitis and hepatosplenomegaly. Babies with microcephaly or hepatitis due to inherited metabolic causes and petechiae due to sepsis were excluded. DNA was extracted from urine samples by Qiagen column kits. Initial PCR screening was done using gB gene primers and the positive samples were subjected to another PCR using primers that amplify the full-length gN gene. The presence of multiple gN genomic variants was determined on 37 PCR positive samples by RFLP, using 15µl of gN PCR amplicon with restriction enzymes Sacl, SalI and Scal (Pignatelli et al., 2003). Results were confirmed by nucleotide sequencing of some strains.

**Results:** Of 100 babies tested, samples from 64 were positive by gB PCR for CMV. Thirty-seven of these samples were amplified by gN PCR and typed by RFLP. gN-1 type was identified in 14 (37.8%), gN-3 in 2 (5.4%) and gN-4 in 17 (45.9%). Multiple gN types was found in 4 (10.8%) of the samples. None of the samples contained gN-2 type.

**Conclusions:** The results of our study showed that gN-4 was the most common gN genotype followed by gN-1. To our knowledge, this is the first study from India on gN genotyping in infants with CMV infection. This information is of value in understanding the molecular epidemiology of circulating CMV strains. An interesting finding of our study is the demonstration that about 10% of children were infected with multiple CMV strains.

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**P-14**  **National surveillance of symptomatic congenital CMV in the UK and Ireland 2001-2002: presentation and outcome.**
Catherine S Peckham, Mike Sharland, Pat A Tookey. Institute of Child Health, University College London, London, UK.

**Background:** The UK birth prevalence of congenital CMV is 3-4/1000 births with ~10% of congenitally infected neonates presenting with CMV-associated symptoms. With the development of oral preparations and less toxic drugs that may reduce subsequent disability, reliable estimates of the burden of disease due to congenital CMV at the population level in the British Isles are required.

**Objectives:** To explore the presentation and management of clinically recognized confirmed congenital CMV in infants born over a two-year period, and ascertain outcome in early childhood.

**Methods:** Active national surveillance through the British Paediatric Surveillance Unit, a well-established pediatric network for the study of rare conditions. Clinicians were asked to report any infant with confirmed or suspected congenital CMV, and complete two pro-formas providing information about presentation, diagnosis and management, and subsequent outcome. Confirmed cases were those with laboratory evidence of congenital CMV infection within 3 weeks of birth.

**Results:** 90 cases were confirmed; 20% were born at <32 weeks, median gestation 37 weeks (range 24-42). 24 were asymptomatic at birth and diagnosed because of maternal infection, ultrasound abnormalities, prematurity etc. 40 presented with typical CMV symptoms, 11 with generalized symptoms of prematurity, 13 with microcephaly and/or growth retardation alone, 2 with unrelated syndromes. 10 deaths were reported (5 at <7 days, 4 by 9 weeks, one at 8 months), including 3 diagnosed at post mortem. 17 children (3 of whom died), received IV gancyclovir. Six children (none treated) were lost to follow up at <3 months. Follow-up information was available for 74 children at median age 18 months (range 3-35 months): 12 had severe disability (multiple problems), 19 moderate (eg bilateral deafness), 11 mild (eg unilateral hearing loss), 32 were developing normally. Outcome related to neonatal presentation: among those with known outcome, 24/37 (65%) presenting with typical CMV symptoms died or had severe/moderate disability, compared with 5/22 (23%) who were asymptomatic at birth.

**Conclusion:** Typical CMV symptoms were reported in less than half the cases; the rest were diagnosed because of prenatal concerns or non-specific signs. Adverse outcomes occurred in all groups, but predominantly in those presenting with typical symptoms or prematurity.
Newborn Screening

P-15 Experience with retrospective diagnosis of congenital CMV from dried blood spots in the UK.
Claire Atkinson, S Luck, P Griffiths, S Walter, M Sharland. Centre for Virology, Hampstead Campus, University College London, London, UK.

Background: Congenital cytomegalovirus (CCMV) is the commonest congenital infection causing long term sensorineural hearing loss and neurological impairment in many infected infants. Establishing the prevalence of CCMV related disease in infants has been hampered by the need for virus detection in a sample taken within the first 3 weeks of life. Tests for CMV DNA in neonatal dried blood spots (DBS) has proved a valid means for retrospective diagnosis of congenital infection (Barbi 2006). Objective To evaluate the introduction of DBS testing as a routine diagnostic test.

Methods: DBS received for diagnostic purposes between 2005-2007 were reviewed. An in house TaqMan Real-Time PCR was used to quantify a target within the CMV glycoprotein B gene. All positive samples were retested to confirm positivity, a second confirmatory PCR targeting CMV UL69 was introduced from 09/2007. Stability of CMV DNA was assessed over a 2 year period by repeat sampling of DBS from replicate cards.

Results: 394 samples were received over the 3 year period (2005-2007) from 3 sources (BPSU,CHIC Study and routine request). The age of samples ranged from 7 days to 19 years (median 2 years). Ages of children testing positive were 18 days to 17 years (median 2 years). There was no relationship between the age of the child at analysis and the viral load detectable on the cards. One false positive was identified, prompting introduction of a second confirmatory PCR. Analysis of replicate DBS revealed no cross contamination between negative cards, but a decline in the viral load over the 2 year period in positive cards. There was a significant difference between the median viral load detected after 18-24 months of storage compared to 0-5 months (Median log 2.3 vs 3.0 respectively; p=0.0007) However CMV was still detectable on DBS after 2 years of storage.

Conclusions: The quantity of CMV DNA in DBS may decrease over time but remains detectable in diagnostic samples up to 17 years of age. False positive results do not occur from prolonged storage in normal conditions, but confirmatory PCR is recommended. A diagnostic algorithm has been developed for retrospective diagnosis of CCMV.

P-16 Targeted CMV testing in infants with hearing loss results in missed opportunities.
Erica Blood, Sandra K. Burchett, Margaret Kenna. Children's Hospital Boston, Boston, MA.

Background: Congenital cytomegalovirus (CMV) infection occurs in up to 2% of live births, and is estimated to be responsible for 10-30% of childhood hearing loss. Although 99% of the infants in our region are screened for hearing loss, there is no standardized CMV testing after a hearing loss diagnosis. Therefore, infants with congenital CMV are being diagnosed late, limiting therapeutic options. We implemented a protocol to offer CMV testing to infants who were diagnosed with hearing loss.

Objectives: To determine the age of confirmed hearing loss and CMV infection status identification in a cohort of infants undergoing initial diagnostic hearing test after a failed newborn hearing screen. To determine if use of this protocol identified CMV infection status at an age when early intervention could improve hearing outcomes.

Methods: Between Sept. 1, 2207 and May 31, 2008 infants <12 months of age who failed a newborn hearing screen and had confirmed hearing loss by auditory brainstem response (ABR) test at Children's Hospital Boston were prospectively identified and offered CMV testing via urine or saliva shell vial culture.

Results: 134 ABR’s were scheduled for infants who did not pass their newborn hearing screen. 70 (52%) of those were scheduled at < 30 days of life, and 102 (76%) at < 8 weeks of life. 56 (42%) were completed at <30 days of life. Of 114 completed ABRs 33 (29%) were abnormal. Of 33 infants with abnormal ABRs, 7 (21%) were CMV tested (4 at <30 days of life).

Conclusions: These results suggest that infants with hearing loss are not being assessed for CMV infection at an age when therapeutic options may be beneficial. Only half of the infants in our cohort with hearing loss were diagnosed at less than 30 days, when early treatment of CMV could decrease the progression of hearing loss. These data illustrate the need for standardized CMV testing algorithms in infants who are diagnosed with hearing loss. CMV testing at confirmed hearing loss may not facilitate the diagnosis of CMV before 30 days of life, and consideration should be given to CMV testing at failed hearing screen.
**P-17 The Contribution of CMV to Hydrocephalus: A Pilot Study Using Newborn Dried Blood Spots.**
Sarah Collier, S Dollard, J Mei, WH Hannon, JL Frias, SA Rasmussen, GM Shaw, R Meyer, S Chaing, M Canfield. Centers for Disease Control and Prevention, Atlanta, GA.

**Background:** Congenital hydrocephalus is characterized by an excess accumulation of cerebrospinal fluid that surrounds the brain and spinal cord and occurs in 1 in 1,250 live born infants. Congenital cytomegalovirus (CMV) infection has been identified as a cause of hydrocephalus, but the contribution of this infection to the occurrence of congenital hydrocephalus has not been well-documented.

**Objectives:** We performed a pilot study to determine whether residual dried blood spots (DBS) could be used to investigate the association between congenital CMV infection, as indicated by the presence of CMV DNA, and hydrocephalus using a case-control study design.

**Methods:** Live-born infants with hydrocephalus (cases) not due to a recognized genetic cause were identified using birth defects surveillance systems in California, North Carolina, and Texas. Infants without major birth defects (controls) were randomly selected from the same geographic area as the cases. After removal of personal identifiers, DBS from case- and control-infants were examined. CMV testing was done without knowledge of case/control status and known positive and negative CMV DBS were added into each set. Presence or absence of CMV infection was assessed using a PCR-based assay.

**Results:** Preliminary results indicated 1.5% of infants with hydrocephalus (6/410) and 0.7% of control-infants (3/448) tested positive for CMV (OR 2.2, 95% CI 0.5-10.8). Among the blinded control specimens in the panel, all negative specimens and 12/13 positive specimens were correctly identified.

**Conclusions:** In this pilot study, evidence of congenital CMV infection was detected in more infants with hydrocephalus than among infants without birth defects, but the estimates were imprecise.

**P-18 Diagnosis of congenital cytomegalovirus infection in the absence of a universal screening program.**
Anna Goncé, O Coll, M López, S Hernández, JM Pérez, MA Marcos, J Bosch, E Gratacós. Department of Maternal-fetal Medicine, Hospital Clinic, University of Barcelona, Barcelona, Spain.

**Background:** Cytomegalovirus (CMV) is the most frequent congenital infection with a high rate of neonatal sequelae but routine screening during pregnancy or in the neonatal period is currently not recommended in Spain.

**Objective:** To determine the incidence of CMV congenital infection with a non-universal screening policy.

**Methods:** Study period: 2006-2007. Maternal CMV serology was selectively determined if the following were present: maternal clinical symptoms or documented exposure, related fetal ultrasound anomalies (CNS/fetal hydrops/hyperecogenic bowel) or soft markers (intrauterine growth restriction (IUGR)/poly/oxygohydramnios). An amniocentesis was performed to determine if CMV DNA-PCR was present in the amniotic fluid (AF). Neonatal screening was selectively assessed (urine viral DNA) in symptomatic newborns, in extreme preterm infants (<32 weeks) or in babies born to HIV infected mothers. An infant was considered infected if CMV DNA was present in AF or in urine within 2 weeks of life.

**Results:** Over a 2 year-period 11 cases of congenital CMV infection were diagnosed with an overall incidence of 1.4% births. Severe ultrasound anomalies compatible with the infection along with a maternal positive serology (IgG or IgG and IgM) was observed in 6 cases and in 5 cases a fetal infection was confirmed (83.3%). Ultrasound soft markers associated with a positive maternal IgM was observed in 8 cases and in 2 of them a congenital infection was confirmed (25%) (fetal hydramnios and IUGR). In the 11 cases where a maternal infection was diagnosed after maternal clinical signs or exposure with a confirmed positive IgM, none had a congenital infection (0%). Four congenital infections were first diagnosed at birth. Two of them were extremely preterm IUGR infants born to a mother with severe preeclampsia and the other two were term babies born to an HIV-infected mother.

**Conclusions:** With the current policy of detection of CMV congenital infection, the presence of specific ultrasound markers and a maternal serological confirmation, the risk of CMV fetal infection is very high. IUGR and newborns from HIV-infected mothers could benefit from a neonatal screening policy. In the absence of ultrasound markers or HIV maternal infection, the rate of symptomatic congenital infection is probably low.
**P-19 Standardization of Neonatal CMV Screening from Dried Blood Spots.**
Mona Shahbazian, Reina Karunaratne, Jerry Boonyaratanakornkit, Alana Benn, Ralf Schonbrunner. AcroMetrix, Benicia, CA.

**Background:** Congenital cytomegalovirus (CMV) infection is seen in ~1% of births. Approximately 20% of cases become symptomatic, most commonly causing deafness. Neonatal CMV screening is essential for early detection and rapid implementation of therapeutic measures. Currently, newborn screening involves the extraction and PCR amplification of CMV DNA from dried blood spots (DBS). For calibration and quantification of viral load, CMV DNA is generally amplified in parallel, but this practice assumes 100% efficiency of nucleic acid extraction from DBS.

**Objective:** To accurately calibrate CMV testing from DBS, a calibrator should follow the same analytic process as patient samples. Our aim was to develop such a calibrator for quantification and standardization of CMV viral load testing in newborns.

**Methods:** We compared the performance of a liquid calibrator consisting of intact CMV virions in plasma to a dry calibrator consisting of 3-mm punches of filter paper onto which CMV in plasma was spotted. DNA extraction for the liquid control was performed using the QIAamp Virus MinElute Kit (QIAGEN) and for the punches was performed using standard thermal extraction methods. Real time PCR was performed using the QIAGEN artus CMV kit.

**Results:** The mean Ct was plotted against the log of absolute number of CMV copies, assuming 100% efficiency of DNA extraction. The slopes of the regression lines for the two calibrators showed approximately equivalent PCR efficiencies- 92% for the liquid calibrator and 94% for the filter paper calibrator. The coefficients of determination indicated a high degree of linearity- 0.99 for the liquid calibrator and 0.97 for the dry calibrator. The intercepts, however, demonstrated a difference of ~3 Cts, or almost one log difference in titer between the two calibrators.

**Conclusion:** We have shown that the use of a liquid calibrator for CMV screening from DBS will cause underestimation of viral load in newborns by approximately one log, and presumably even more if a DNA-based calibrator is used. This could have a significant impact for clinical decisions, particularly when CMV titer is low. We suggest using dried CMV plasma spots as a more accurate calibrator that would help standardize neonatal CMV screening.

**P-20 Diagnosis of congenital CMV infection in dried blood spots (DBS) : the French experience.**
Christelle Vauloup-Fellous, Sophie Couderc, Magny Jean-François, Sophie Parat, Sandrine Marlin, Vincent Couloigner, Natalie Loundon, Marianne Leruez-Ville. Service de Microbiologie-Immunologie biologique, Hospital Antoine Béclere, University Paris-Sudart, Clamart, France.

**Background:** In the absence of congenital CMV screening policy, detection of CMV DNA in DBS (Guthrie cards) has been developed for retrospective diagnosis in children with hearing loss.

**Objectives:** 1) To evaluate specificity and sensitivity of CMV DNA detection in DBS for diagnosis of congenital infection in comparison to the gold standard method (CMV detection in urine) 2) To retrospectively diagnose CMV congenital infection in DBS in a cohort of children with hearing loss.

**Methods:** The study population consisted of: 1) 175 neonates who had a CMV congenital infection diagnosis done by PCR or culture in the urine collected in the first week of life. Thirty of these neonates had positive CMV detection in their urine. 2) 57 children diagnosed with hearing loss. CMV DNA was detected in the Guthrie cards by 2 methods. Method 1 consisted of DNA extraction in a whole DBS with NaOH lysis followed by QIAamp DNA Blood Mini Kit and amplification by an in house real time PCR in duplicate. Method 2 was a phenol/chloroform extraction of a whole DBS followed by amplification with the CMV PCR kit (Abbott, France).

**Results:** Sensitivity and specificity of method 1 were of 100% (38/38) and 97% (133/137) respectively when at least one duplicate was positive. Sensitivity and specificity of method 2 were 94% (32/34) and 96.8% (123/127) respectively. Median viral loads in discordant cases (negative detection in urine and positive PCR in DBS) were 400 [35-6,000] and 38 [9-500] copies/ml with method 1 and method 2 respectively. CMV PCR was positive in 8 of the 57 children with hearing loss. Both PCR assays were positive in 7 cases, in 1 case only method 1 could be realized. The median CMV DNA load in these 8 cases was 24,690 [1,300-100,000] copies/ml with method 1.

**Conclusions:** Sensitivity and specificity of CMV DNA detection in DBS with either method allow retrospective diagnosis of hearing loss in children.
Pathogenesis and Immunology

P-21  
**HCMV Altered Gene Expression in the Placenta and Brain: Implications for Congenital Disease.**
Donald J. Alcendor, Charmaine O’Brien, Wen Qin Zhu. Meharry Medical College, Center for AIDS Health Disparities Research and Department of Microbial Pathogenesis and Immune Response, Nashville, TN.

**Background:** Human cytomegalovirus (HCMV) is a ubiquitous pathogen and is the most common infectious cause of congenital disease in children. In the United States alone, 40,000 children are born each year with congenital CMV infection. CMV is the leading infectious cause of mental retardation and deafness in children. Primary cytomegalovirus infection in utero is second only to Down syndrome in causing birth defects. Central nervous system abnormalities in newborn babies can include vision loss, mental retardation, motor deficits, seizures, and hearing loss. It will also cause severe disease in immunocompromised patients such as those with HIV. These children will often experience extended hospital stays and require more aggressive and long-term health care measures for support.

**Methods:** To study gene dyregulations in the placenta and brain we have performed gene array analysis on mock and HCMV infected primary human cytotrophoblasts and human cerebral vascular pericytes from the brain cortex. In addition, we have developed an in vitro Tri-cell culture model of the blood brain-barrier (BBB) made up of astrocytes, brain microvascular endothelial cells (BMVEC) and pericytes. We have employed RT-PCR and dual labeled immunohistochemical staining of archival placental tissue for in vivo validation of expression profiles observed in gene arrays.

**Results:** Both cytotrophoblasts and brain vascular pericytes are fully permissive for HCMV infection. Morphological appearance of our culture model has unique features that are different from the individual cell types. Microarray analysis has identified genes altered after HCMV infection of cytotrophoblasts and brain pericytes that includes genes associated with neuronal injury. We have established dual label immunohistochemical staining of cellular and viral antigens in archival placental tissue from infants with CMV associated congenital disease.

**Conclusions:** We have identified a number of dysregulated genes in cytotrophoblasts and brain pericytes after HCMV infection which may serve as disease biomarkers or potential therapeutic targets. Our ongoing studies will be to test our in vitro BBB model after HCMV infection and follow virus dissemination in real time by time-lapse microscopy. Understanding molecular targets of viral pathogenesis in the brain and placenta will aid in elucidation of mechanisms involved in CMV associated congenital disease.

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P-22  
**CMV infection in early and term placental villi : implications for indoleamine 2,3-dioxygenase.**
Charlotte Casper, Helene Lopez, Michel Baron, Talal Al Saati, Isabelle Duga Neulat, Alain Berrebi, Christelle Cristini, Monique Plantavid. U563 and Children’s Hospital, Toulouse, France.

**Background:** Human cytomegalovirus (CMV) is the most common cause of viral intrauterine infection in developed countries. Placental infection in utero suggest hematogenous spread from the mother. Indoleamine 2,3-dioxygenase (IDO) is highly expressed in the placenta. IDO activity is known to suppress T cell activation by degrading tryptophan, controlling the maternal immune response. and modify the outcome of the pregnancy.

**Objective:** To evaluate a new ex vivo model of long term placental histocultures in order to investigate the permissivity of early and term placenta to CMV. To investigated the activity profile of indolamine2,3 dioxygenase (IDO) during CMV infection.

**Methods:** Ten first trimester placenta were obtained following elective abortion and ten term placenta were obtained after elective caesarean section. Two methods of culture were established. Fresh placental chorionic villi were isolated, washed and distributed on collagen sponge gels as previously described (Faye, Placenta 2005). Endotheliotropic strain VHL/E was left in contact with the microexplants overnight (Method A). The culture medium was collected and fresh medium added on days 1, 5, 10, 15, 21. In method B, villi blocks were distributed on sponge gels in plates covered with subconfluent VHL/E infected layers of MRC5. After 5 days, the sponge gels were transferred to plates with fresh uninfected medium only. The culture medium was collected and fresh medium added on day 6, 10, 15 and 21. Viral plaque assay in MRC5 cells was performed on each collection day. Tissue was fixed and embedded in paraffin for histopathological and immunohistochemical study with detection of immediate early antigen (IEA). Constitutive and INF-γ inducible IDO activity was quantified by measuring kynurenine production in the histoculture supernatant by colorimetry.

**Results:** Viral infection could be seen in tissue sections of infected villi blocks after day 10 for method A in early placenta but remained negative in term placenta. Viral plaque assay showed a productive and increasing replication in supernatants at days 15 and 21 for early placenta (mean 6200 PFU/mL) but remained negative for term placenta. Method B was efficient both for infection of early and term placenta. The upregulated IDO activity by INF-γ was suppressed by CMV in early and term placenta.
**P-23 Viral and immunological analysis in CMV breakthrough disease in a transplant patient.**
Jaythoon Hassan, Kirsten Schaffer, Julie Moran, Margaret Duffy, Aidan McCormack, William Hall. National Virus Reference Laboratory, Dublin, Ireland.

We report a case of CMV breakthrough disease in a D+/R- liver transplant patient while being on valganciclovir prophylaxis. CMV viral load, 6250 copies/ml, was first detected at 50 days post-transplant. The virally encoded protein UL97 was sequenced and codons 460-607 were analyzed. A deletion at 594-595 and C603W mutation was detected which is known to confer resistance to ganciclovir. At day 121, CMV viremia reached a peak of 59,400 copies/ml and foscarnet was commenced. Viral loads remained below 10,000 copies/ml when the white cell count exceeded 6000/l and the lymphocyte count was greater than 1000/l. Lymphocyte subset analysis at days 136 and 300 showed a change in the CD4+/CD8+ ratio from 3 to 1. Analysis of the differentiation phenotype showed that the CD3+CD8+ cells were mainly terminal effector memory cells (CD45RA+ CCR7+) whereas the CD3+CD4+ cells were largely naive (CD45RA+ CCR7+) and central memory cells (CD45RA- CCR7+). Seroconversion was delayed to day 230. Circulating levels of IL-10 correlated significantly with viral load (p<0.03). These findings offer a unique insight into monitoring the kinetics of uncontrolled viral replication and viral clearance in an immunosuppressed host.

**Conclusion:** This is the first long term ex vivo placental model of histocultures with effective CMV production. Our findings suggest that early placenta are more permissive to CMV infection than term placenta. The functional consequence of inhibition of IDO activity with CMV infection could be a global placental dysfunction.

**P-24 In Vitro Model of CMV-induced Embryonic Cochlear Pathogenesis.**
Tina Jaskoll, Michael Melnick. Laboratory Developmental Genetics, University of Southern California, Los Angeles, CA.

Congenital cytomegalovirus (CMV) infection is a major cause of sensorineural hearing loss (SNHL) in children. It is estimated that at least 8000 infants born annually in the US will have congenital CMV-induced SNHL, with a significant proportion of these children exhibiting delayed onset and progressive deterioration of hearing after the newborn period. The fact that in utero CMV infection causes SNHL, whereas postnatal infection does not, suggests that CMV induces unique changes in embryonic signaling pathways. We postulate that the pathogenesis of CMV-induced SNHL begins in utero and is likely mediated through virally-induced dysregulation of signaling pathways during early and later stages of inner ear morphogenesis. Presently, the mechanism underlying congenital CMV-induced SNHL is poorly understood since in vivo rodent models are of limited utility due to the inability of CMV to cross the placenta of mice and rats. Our objective is to develop a new in vitro model to delineate the mechanisms underlying CMV-induced cochlear pathogenesis, as well as for screening new drugs to preclude CMV-induced pathology. We developed a mouse embryonic organ culture system to study the effect of mouse CMV (mCMV) infection on cochlear morphogenesis. Infection of E15 mouse cochlea with mCMV for 9-13 days in vitro resulted in abnormal cells in the organ of Corti, Reissner’s membrane, stria vascularis, scala tympani and scala vestibule. The histopathology and viral distribution in mCMV-infected cultured mouse embryonic cochlea are similar to those seen in children with congenital CMV infection. Our results suggest that this embryonic mouse cochlear culture system models the inner ear pathology seen in CMV-infected infants and allows us to closely investigate the mechanism underlying CMV-induced cochlear pathogenesis in vivo. Importantly, our observation that treatment with the antiviral, acyclovir, rescues the abnormal phenotype and restores it to normal suggests that our in vitro mammalian model is a good system for screening new anti-CMV drugs, including new antivirals. This is clinically important since the prevalence of CMV-induced SNHL is considerable, present anti-viral therapies are teratogenic in themselves, and long-term administration of antiviral drugs to infected infants presents serious safety concerns.

**P-25 Cytomegalovirus strain diversity in infected fetuses: cloning analysis of the glycoprotein B gene.**

**Background:** Infections with multiple CMV strains of different genotypes have been shown to occur in immunocompromised and immunocompetent individuals as well as in infected fetuses. It was suggested that multiple strains infections might be more severe. Whether multiple strains are acquired through a single infection with codisseminating genotypes or through consecutive superinfection over time is not well known.

**Objectives:** To study CMV strain diversity in samples obtained from infected fetuses using a cloning method.

**Methods:** 16 samples obtained from 10 infected fetuses (10 amniotic fluids, 3 fetal blood, 2 urine sample at birth and 1 ascitic fluid) were studied. All cases but 1 were secondary to a primary maternal infection. Two fetal infections were asymptomatic (cases 5 and 6), 7 fetuses had ultrasounds abnormalities including neurologic involvement in 3 cases. Sequential samples at different time of gestation were evaluated (> 6 weeks interval) in 3 cases. DNA was extracted from samples and subjected to PCR to amplify the cleavage region of the gB gene (region between: 1319-1604). PCR products were directly cloned into the PGEM-T easy Vector system (Promega). For each sample, 20 clones were sequenced bidirectionally. Nucleotide sequences were aligned with Clustal W1.6 for phylogenetic analysis (PHILIP package).
Results: Genotypes 1, 2, 3 and undetermined were found in 6, 2 and 1 cases respectively. Each fetus harbored a unique gB genotype. However, sequence variability was demonstrated in 10 samples (8 cases) with the presence of 1 to 5 minor clones. Nucleotides diversity displayed between major and minor clones was low ranging from 0.3 to 0.6% (1 or 2 nucleotides mutations). When amniotic fluid and blood samples were analyzed on the same day, the same major variant was found in both compartments. Analysis of sequential samples demonstrated that major clones remain identical and that minor clones either persist (cases 5 and 6) or change (case 1) over time.

Conclusions: In this study, only one genotype variant could be found in each fetus. Cloning showed a mixture of major and minor variants of the same genotype in most samples. There was no codissemination of multiple genotypes in fetal primary infection.

P-26 Peptide-Based Inhibition of HCMV through Blocking Virus: Cell Membrane Fusion.
Lilia I. Melnik, Robert F. Garry. Tulane University Health Sciences Center, New Orleans, LA.

Congenital infection occurs in about 3% of newborns in the United States and Europe annually and can result in Congenital CMV Syndrome with severe neurological and cognitive disorders. The currently available treatments for HCMV are ganciclovir and foscarnet. Both of these drugs are inhibitors of viral replication, but can cause various side effects. The aim of this study is to develop peptides that specifically inhibit the fusion of HCMV to the host cell membrane as a novel approach to prevent HCMV infection. Glycoprotein B (gB) is a major glycoprotein of HCMV that is highly conserved across the herpes virus family. Using the Wimley and White interfacial hydrophobicity scale (IHS), we identified several regions within gB displaying a high propensity to interact with the lipid surface of cell membranes. Next, we synthesized several peptides that are analogous to identified regions of gB. The inhibitory effect of each peptide was evaluated by infecting human foreskin fibroblasts (HFF) and human extravillous cytotrophoblasts (SGHPL-4) with the Towne GFP strain of HCMV (0.5 MOI) preincubated with a range of concentrations of inhibitory peptides at 37°C for 90 minutes. GFP positive cells were visualized 48 hours post infection by fluorescence microscopy and analyzed by Flow Cytometry. The ability of the peptides to block the HCMV inhibition of invasion will be tested using FluoroBlok assays. The mechanism by which these peptides block HCMV infection will be determined by fusion assays. Peptides were tested at the following concentrations: 100uM, 50uM, 25uM, 10uM, 5uM, 2.5uM, 1.25uM, 0.625uM, and 0.3125uM, 0.156uM, and 0.078uM. One of the peptides displayed 100% inhibition at the concentration of 100uM and 50uM. All of the peptides tested displayed very different inhibition curves indicating that each of them possesses distinct biophysical properties. The combination of two of the peptides showed greater than 50% inhibition of HCMV infection at the concentration of 0.125uM each. Peptides designed to target the fusogenic domains of gB provide a potential basis for the development of novel therapeutic interference for HCMV as well as other herpesviruses.

P-27 The quality of the maternal humoral immune responses to CMV Modulate viral infection in the placenta.
Naoki Nozawa, June Fang-Hoover, Ekaterina Maidji, Susan McDonagh, Lenore Pereira. Department of Cell and Tissue Biology, University of California, San Francisco, CA.

Background: CMV is the most common congenital viral infection. Approximately 40% of pregnant woman with primary infection transmit virus to their babies, but only 2% with preconceptual immunity. CMV DNA and replication proteins were found often in placentas of women with low-avidity IgG, whereas infection was suppressed with high-avidity IgG. These results suggest that maternal immunity modulate transplacental transmission.

Objectives: Determine whether CMV-infected placentas correlate with low-avidity maternal IgG.

Methods: Clinical samples include placentas at delivery, placental (maternal) and cord (fetal) blood samples obtained from UCSF Moffitt Hospital. Placental biopsy specimens and blood specimens were tested for CMV DNA by conventional PCR using primer sets for the immediate early gene and glycoprotein B. CMV IgG avidity was quantified using Radim ELISAs (Radim Diagnostics) and specific IgG analyzed by immunoblot with recombinant CMV proteins (recomBlot, Microgen).

Results: Among eight mother-infant pairs with high-avidity CMV-specific IgG we found equal or higher levels in cord blood as compared with maternal circulation. Two pairs with moderate titers had somewhat less. Among three mother-infant pairs with low-avidity IgG we failed to detect CMV-specific IgG in cord blood samples. Immunoblot analysis showed patterns consistent with past and recurrent CMV infections in paired sera from the group with high-avidity IgG and potent neutralizing antibodies reactive with immunogenic viral proteins (i.e., p150, gB1, and gB2). Several sera also reacted with IE proteins, suggesting reactivated infection that was supported by detectable CMV DNA in placental biopsy specimens. In contrast, low-avidity CMV-specific IgG was weakly reactive with viral proteins.
Conclusions: The results suggest that high-avidity CMV-specific antibodies reach a threshold in maternal circulation and accumulate in fetal blood by transcytosis of the neonatal Fc receptor across syncytiotrophoblasts. The pattern in strongly-immune seropositive women with high-avidity IgG and low levels of CMV DNA in placentas, suggests viral reactivation and clearance of immune complexes without fetal infection. Women with low-avidity IgG and low neutralizing activity reactive with few, if any, CMV proteins by immunoblot have little or no protection during primary infection, resulting in viral replication and dissemination in the fetus.

P-28 Mechanisms of Transmission of Cytomegalovirus from Mother to Baby.
William D. Rawlinson, Jonathan Howard, Beverley Hall, Sharon S. W. Chow, Cheryl A. Jones, Nicole Graf, Susan Arbuckle. School of Medical Sciences, University of New South Wales, Virology Division, POWH & UNSW Research Laboratories, Randwick, Australia.

Background: Placental viral transmission is now well documented for human cytomegalovirus (CMV) and more recently for a number of other viruses. Symptomatic congenital CMV infection affects 300-500 neonates in Australia per year, 10% of whom will develop severe disease and long-term impairment. Many pregnancies result in miscarriage or fetal death and abnormalities where the cause is never known.

Objective: The primary aim is to determine cellular and viral processes that facilitate CMV migration from mother to fetus.

Retrospective Cohort: We enrolled 195 stillbirths undergoing autopsy at Children's Hospital at Westmead (Australia) during 2005-2006, with no cause found after autopsy in 65% (127) cases. Cases were defined as ≥32 weeks gestation, singletons, with no birth defect demonstrated at routine post mortem, and no bacterial pathogen demonstrated on routine microbiological culture. Experimental method: Total nucleic acid was extracted from formalin-fixed specimens of placenta, liver and kidney, and from the 100 available newborn screening cards (NBSC) taken at cardiac puncture prior to autopsy. Using multiplex PCR (mPCR)(1), we screened CMV and other infectious agents. In-situ PCR (IS-PCR) was then performed on the CMV positive placenta. We measured mid-trimester amniotic fluid cytokine levels using 27-plex Human Cytokine Assay (BioRad).

Results: To date, we screened 130 stillbirths for 22 pathogens and we detected CMV (35%) and other infectious agents in placenta, liver, kidney and NBSC. Other infectious agents including HHV-6, -7 and -8, were also detected in some of the CMV-infected stillbirths. Using our published in situ PCR, we observed that CMV infection was predominantly localized in the endothelial cells of infected tissue and placental syncytiotrophoblasts(2). The levels of the cytokines IP-10 and IL-1ra (pro-inflammatory markers) were significantly elevated (p<0.001) in the amniotic fluids examined.

Conclusions: This study provides data on the pathogenesis of CMV in stillbirths. We see association of stillbirth with CMV and other pathogens, with accompanying significant increase in some pro-inflammatory cytokines. (1) McIver et al. (2005) J Clin Micro 43 (10): 5102-5110. (2) Trincado et al. (2005) J Infect Dis 192: 650-657.

P-29 Neutralizing Antibody to Wild Human Cytomegalovirus Strain VR1814 Do Not Prevent Fetal Infection.
Maria Grazia Revello, Antonella Sarasini, Giuseppe Gerna. Servizio di Virologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

Background: Administration of human cytomegalovirus (HCMV) hyperimmune globulin (HIG) has been reported to be effective in reducing intrauterine transmission rate when administered to pregnant women with primary HCMV infection. The specific preventive effect has been claimed to be due to, among other factors, the presence in HIG preparations of HCMV neutralizing (Nt) antibody (Ab) at a high titer (Nigro G et al., New Engl J Med 353: 1350-62, 2005). Recently, monoclonal Ab to the 131-128 HCMV locus have been shown to inhibit plaque formation, leukocyte transfer and endothelial cell infection of prototype HCMV clinical strain VR1814 in vitro. Moreover, and contrary to Nt Ab response to the laboratory strain AD169 on fibroblast cells, a strong Nt Ab response to VR1814 has been shown to develop on human umbilical vein endothelial cells (HUVEC) very early after the onset of a primary HCMV infection in vivo (Gerna G et al., J Gen Virol 89: 853-65, 2008). These findings might provide a scientific explanation for the reported efficacy of HCMV HIG.

Materials and Methods: Altogether, 187 sequential sera from 40 pregnant women with documented primary HCMV infection were tested for Nt Ab to VR1814 on HUVEC and to AD169 on fibroblast cells. HCMV-specific IgG, IgM, IgG avidity and DNAemia were also determined. Serum and blood samples collected at ≤60 and >60 days after the onset were analyzed separately for each parameter in both transmitter (n=17) and non-transmitter (n=23) mothers.

Results: No significant difference in qualitative or quantitative Nt Ab response to VR1814 or AD169 was observed in serum samples collected from transmitter or non-transmitter mothers at each time interval. Similarly, no significant difference was observed for the other parameters investigated.
Conclusions: In this limited series of cases, intrauterine HCMV transmission appeared to be unrelated to the presence or amount of either type of Nt Ab in maternal serum samples.

**P-30 Human cytomegalovirus induces HLA-E expression in kidney and mammary epithelial cells.**
Nicolas Twite, Graciela Andrei, Robert Snoeck, Michel Goldman, Arnaud Marchant. Institute for Medical Immunology, Free University of Brussels, Grosselies, Belgium.

**Background:** Urine and breast milk are common routes of human cytomegalovirus (HCMV) transmission. The immune mechanisms controlling the replication of HCMV in the kidney and mammary gland are poorly understood. Transfection of HCMV UL-40 gene into human cell lines (HeLa and HEK-293T) was shown to induce the expression of HLA-E, a ligand for the CD94/NKG2A inhibitory receptor expressed by NK cells and CD8 T lymphocytes.

**Objectives:** The objective of the study is to characterize the influence of HCMV infection on the expression of immunomodulatory molecule HLA-E in kidney and mammary epithelial cells.

**Methods:** Primary mammary and kidney epithelial cells as well as human foreskin fibroblasts were infected with HCMV (AD169 and TB40/E strains) for various periods of time before measuring the membranous expression of classical HLA class I and non-classical HLA-E molecules by flow cytometry. Cell infection was detected by measuring the intracellular expression of IE antigen (68-72 kDa) by flow cytometry and titration in supernatants and cells.

**Results:** Fibroblasts, kidney and mammary epithelial cells were permissive to productive HCMV infection. HCMV infection downregulated classical HLA class I expression in all three cell types. No HLA-E expression was detected at the surface of fibroblasts infected with AD169 or TB40/E strains. In contrast, about 20% infected epithelial cells expressed HLA-E. Peak expression of HLA-E was observed 3 days after infection.

**Conclusions:** These results indicate that mammary and kidney epithelial cells are more susceptible to the induction of HLA-E expression by HCMV than fibroblasts. This phenomenon may have an important influence on the control of HCMV replication by CD94/NKG2A+ cells.

**P-31 Altered Chemokine Signaling in Early Infection of Cytotrophoblasts by Human Cytomegalovirus.**
Jessica Warner, Seth Coffelt, Kenneth Swan, Kerstin Honer zu Bentrup, Deborah Sullivan, Gabriella Pridjian, Cindy Morris. Tulane University School of Medicine, New Orleans, LA.

**Objective:** Primary human cytomegalovirus (HCMV) infection during pregnancy can have devastating consequences for both the mother and fetus. HCMV infection has been implicated in the development of pre-eclampsia and intrauterine growth retardation (IUGR), as well as congenital CMV syndrome in newborns exposed in utero. Previously, we have shown that HCMV infection of cytotrophoblasts inhibits their normal invasion, proliferation, and migration. However, the mechanisms occurring during early establishment of placental infection are largely unknown. We sought to quantify changes in chemokine production and function in early infection of cytotrophoblasts by HCMV.

**Methods:** We assessed the impact of HCMV infection on cytotrophoblasts by performing Luminex-based assays for various cytokines and cellular growth factors. Additionally, transcriptional changes were assessed using real-time PCR. Localization of chemokines and chemokine receptors was determined using immunofluorescence, and total cellular protein levels were examined by flow cytometry. Functional migration of trophoblasts toward chemokines SDF-1 and Gro-α was determined by Fluoroblok migration and invasion assays.

**Results:** We detected significant cytokine dysregulation at both 24 and 48 hours after in vitro HCMV infection of cytotrophoblast cells. Soluble cytokines involved in recruitment of monocytes and macrophages (Gro-α, MCP-3) were downregulated at both 24 and 48 hours after infection. SDF-1 (CXCL12), which is chemotactic for lymphocytes during early inflammation, and has also been shown to promote trophoblast survival and proliferation, was also significantly decreased. By immunofluorescence, we confirmed that both SDF-1 and Gro-α are sequestered intracellularly after HCMV infection. Real-time PCR analysis of these analytes indicates that as early as 24 hours post infection, chemokine profiles are altered. Finally, migration of trophoblasts toward chemokines SDF-1 and Gro-α was determined by Fluoroblok migration and invasion assays.

**Conclusions:** These results suggest that recruitment of cells involved in the anti-viral immune response is being interrupted early in the course of HCMV infection. Additionally, several chemokines important for normal trophoblast function are affected, which may indicate one route by which HCMV impedes trophoblast invasion and migration. Further studies will determine the mechanism of chemokine sequestration, which may involve viral encoded receptors or modulation of endogenous host chemokine receptor expression.
**Postnatal Diagnosis, Treatment, and Follow-up**

**P-32 Ganciclovir treatment of congenital cytomegalovirus infection in newborns.**

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**Background:** Ganciclovir (GCV) is the drug used in the treatment of symptomatic congenital cytomegalovirus infection in the newborns. Treatment of newborns with ganciclovir has limits because of its potential side effects.

**Objective:** The aim of the study was to evaluate the safety of ganciclovir therapy in newborns with congenital cytomegalovirus disease.

**Methods:** Neonates with symptomatic congenital CMV disease were assigned to receive treatment with intravenous ganciclovir. The dose of GCV at start point was 5 mg/kg per dose administered every 12 hours. GCV blood levels were analyzed with HPLC method and the individual doses of GCV depended on the drug serum concentration were used. During and after GCV treatment selected hematological and biochemical parameters of the blood were performed.

**Results:** A total of 57 newborns, hospitalized between January 2002 and December 2006, treated with GCV, were analyzed. In the study group there were 47 (82.46%) term newborns and 10 (17.54%) premature babies (~37 weeks). The mean duration of therapy was 21 (13-44) days. The GCV (median) serum concentration was 6.13 (3.31-8.67) mcg/ml at 0 hour (C max) and 1.51 (0.6-3.04) mcg/ml at 4.5 hours (C 4.5 max) after GCV infusion, respectively. The mean daily dose of GCV was 12.28 ± 4.82 mg/kg/day. The drug was administered every 12 hours in 24 (42.11%) newborns and every 8 hours in 33 (57.89%) newborns. In 36 (63.16%) newborns the doses were increased to obtain appropriate therapeutic levels of the drug in the blood. The following side effects were noted during the GCV treatment: neutropenia in 19 (33.33%), anemia in 30 (52.63%), elevated aminotransferase levels in 8 (14.04%) newborns. No thrombocytopenia was observed. Renal function during and after treatment was normal.

**Conclusions:** 1. GCV is well tolerated in newborns with congenital cytomegalovirus disease, both mature and premature, if its serum concentration monitoring and its individual doses are used. Drug serum concentration monitoring is condition to safety of treatment these group of patients. 2. There is no correlation between individual dose used, prolonged therapy of GCV and observed side effects. Standard formula for GCV treatment is not possible due to individual variability of pharmacokinetic parameters in newborns.

**P-33 Dynamics of compartmentalized ganciclovir-resistance in symptomatic congenital CMV-infection.**

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**Background:** Postnatal antiviral treatment of congenital CMV infection is performed by iv administration of the nucleoside analogon ganciclovir (GCV) in an off label use to reduce neurological sequelae especially hearing loss.

**Objective:** Molecular analysis to detect the reason of treatment failure with persistent viral load in blood during long-term GCV administration.

**Methods:** CMV drug resistance screening was performed from specimens from different body sites using UL97 RFLP analysis and sequencing of the viral UL97/UL54 genes. In vitro long-term propagation of viral strains was done for analysis of the stability of UL97 mutations. The influence of GCV therapy interruption was studied in context of the viral dynamics of ex vivo UL97 wild type and mutant strains.

**Results:** The term infant (BW 3215g) presented with classical symptoms of congenital CMV infection. Virus was detected in blood, urine and CSF. A first GCV treatment cycle was initiated on day 2 p.p. and finished on day 135 p.p. After cessation of the first GCV cycle disseminated CMV infection with chorioretinitis persisted during the first year of life, while pneumonia, hepatitis and encephalitis resolved. In leukocytes no mutation of the UL97 gene was found. A second course of GCV treatment was started at the age of 18 months. During the second GCV cycle a screening of different specimens from blood, throat and eye revealed the emergence of a UL97 C607Y mutation conferring for GCV resistance. Interestingly, in eye swabs only the mutant C607Y strain was found, while in blood only wild type was present. In urine and throat swabs mixed viral strains were found. The second cycle was finished with oral valganciclovir. At the age of 2 years only UL97 wild type was detectable.

**Conclusion:** To our knowledge this is the first report on the emergence of a UL97 mutation conferring for GCV resistance in congenital CMV infection. The compartment-specific distribution of mutant viral strains outside blood has strong implications for genotypic drug resistance diagnosis. The CMV screening of congenital infected infants using corneal swabs offers new options for molecular analysis of CMV compartments in vivo.
P-34  **CMV Association in Extrahepatic Biliary Atresia: Awareness Needed for Prevention and Early Treatment.**
Vineeta Khare, Prashant Gupta, Janak Kishore. Department of Microbiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India.

**Background:** In India, serological surveys have shown the prevalence of CMV antibodies in adult population to be about 80-90%. Cytomegalovirus (CMV) infection has been implicated in the causation of extrahepatic biliary atresia (EHBA) in neonates born to CMV infected females. Incidence of EHBA in newborns of CMV infected females is not known in India. Treatment is surgical and prognosis is good only when it is done before the infant attain the age of 8 weeks thus requiring early referral.

**Objectives:** To determine the frequency with which patients with EHBA are infected with CMV and to ascertain the age at referral to a specialty centre for surgical correction of EHBA.

**Methods:** 74 cases of biopsy proven EHBA were managed at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow from January 2005 through May 2008. IgM antibody detection in serum for CMV was done using ELISA kit. The data analyzed included age, sex, clinical presentation, age at referral, Liver function tests, urine examination, abdominal ultrasonography, hepatobiliary scintigraphy, liver biopsy, management, complications and follow-up.

**Results:** Out of 74 EHBA cases, 53 were male and 21 female. 22 (29.7%) were positive for IgM antibodies for CMV. Age of onset of jaundice ranged from since birth to 60 days. The mean age at referral was 14 weeks (range 4-32 weeks). Kasai Portoenterostomy (PE) was performed in 19 infants and the mean age at surgery was 15 weeks (range 4 to 36 weeks). 54 children could not undergo Kasai PE because either they were more than 20 weeks old or presented with extensive liver cirrhosis or due to financial constraints of parents. 1 patient died.

**Conclusions:** CMV infection was present in 29.7% of patients with EHBA at the time of referral. Although jaundice, pale stools and dark urine were observed in early infancy, referral was always late. Seeing CMV association in EHBA, awareness programs among Indian pregnant females for prevention of CMV infection are necessary and possibility of EHBA must be kept in mind when evaluating jaundiced infants born to CMV positive females.

P-35  **Evidence of sub-therapeutic concentrations in a pediatric population receiving ganciclovir.**

**Background:** Ganciclovir (GCV) may be commenced in infants with congenitally or postnatally acquired cytomegalovirus (CMV) infection and in immunocompromised patients who become viraemic. The dose of IV GCV recommended in the children’s British National Formulary is 5mg/kg twice daily, although higher doses have been studied for treatment of congenital CMV. Doses of oral valganciclovir used vary even more widely. Reference ranges are often quoted for adults but some of the evidence on which these are based is contentious and how these relate to treatment and viral suppression in infants is unknown.

**Objectives:** To evaluate GCV levels from specimens received for routine diagnostic purposes by a national reference laboratory in the United Kingdom (UK).

**Methods:** Serum GCV levels (reported in mg/L) received by the UK Antimicrobial Reference laboratory in Bristol from 01/11/1999 to 31/3/2007 were reviewed. GCV levels from samples received from different pediatric age groups were analyzed and compared to those obtained from adults (individuals more than 18 years of age).

**Results:** 95 specimens were received from 32 patients aged < 6 months (28% of all specimens received and 25% of all patients). The median trough level seen in infants aged <6 months was significantly less than that seen in adults (0.35 vs 2.1 P = <0.0001) and there were significantly more levels that were <0.5mg/L in the younger age groups. Median peak levels from those aged < 6 months were significantly lower than those from adults (4.8 vs 5.65 P=0.047). Very few patients in any age group achieved peak levels >7.0 mg/L (the lower end of the reference range quoted by some laboratories) and 20-35% of peak values from all age groups were <3.0mg/L.

**Conclusions:** The high proportion of children with trough levels <0.5mg/L shows significant underdosing in these younger age groups. This could be of concern if prolonged treatment courses are considered for babies with congenital CMV. Information correlating peak and trough levels with maximal viral suppression in vivo are needed before rational treatment decisions can be made. In view of our data it is important that levels are always interpreted as part of a fuller clinical picture.

P-36  **PCR Analysis of Archived Blood Spots Demonstrates Congenital CMV is a Major Cause of Hearing Loss.**
Bazak Sharon, K. Yeon Choi, Lisa A Schimmenti, Frank L Rimell, Mark McCann, Mark R. Schleiss. University of Minnesota, Minneapolis, MN.
Background: Cytomegalovirus (CMV), a common congenital infection is present in 0.5-2% of all newborns. Irrespective of the presence or absence of symptoms in the newborn period, children with congenital CMV infection have a significant risk to develop later sequelae most commonly sensorineural hearing loss (SNHL). The diagnosis of congenital CMV beyond the neonatal period is challenging insofar as definitive diagnosis can only be made by demonstrating the virus in samples obtained in the first 3 weeks of life. Novel molecular techniques and the availability of newborn blood spots (NBS) have created the opportunity for retrospective diagnosis.

Objectives: To test the hypotheses that: 1) Congenital CMV infection can be retrospectively diagnosed by molecular analysis of DNA extracted from archived NBS. 2) That the prevalence of congenital CMV infection in children with SNHL is significantly higher than the background rate of 0.5-2%.

Design/Methods: The cohort consisted of children with SNHL referred to the University of Minnesota. Based on evaluation by a geneticist and an otolaryngologist patients were further divided into those with or without a genetic cause. Following IRB approval and with parental consent, NBS of enrolled children were retrieved from the Minnesota Department of Health (MDH) and analyzed for CMV genome by real-time PCR.

Results: Twenty nine children with hearing impairment have been enrolled, from which 11 had a genetic etiology for their SNHL (37%). The NBS of all subjects have been retrieved from the MDH for PCR analysis. Nine of the 29 enrolled children were diagnosed with congenital CMV infection (30%). CMV genome was found in 8 out of the 19 children who had no identifiable or suspected genetic etiology (42%), while one child with congenital CMV infection was also diagnosed with long QT syndrome.

Conclusions: Congenital CMV infection can be diagnosed retrospectively using archived NBS. Our study shows that congenital CMV infection is found in one in four children with SNHL referred to a university-based clinic. Furthermore, we found that 42% of children with SNHL without a genetic etiology were born with CMV infection demonstrating that this infection is a leading cause for acquired childhood hearing deficit.

P-37 Physical and intellectual development in children with CMV asymptomatic congenital infection.

Xinwen Zhang, Fen Li. MCH faculty of Medical College of XJTU, Xi’an, China.

Background: Although about 90% of congenital cytomegalovirus (CMV) infection is asymptomatic in newborn, some of them could show sequelae later in life. Qinba mountain area is a place with high incidence of mental retardation and a high rate of CMV intrauterine transmission in China. The correlation between asymptomatic congenital CMV infection and developmental outcomes of children in this area remain unclear.

Objectives: To investigate the impact of asymptomatic congenital CMV infection on physical and intellectual development of children during the first 6 years of life in Qinba mountain area.

Methods: Longitudinal cohort study. 49 of all the 54 children with asymptomatic congenital CMV infection, who were born in Qinba mountain area during the 4-year period from January 1997 to December 2000, were followed prospectively in a study for surveying physical growth and intellectual developments. Head circumference, length and weight were used to assess physical growth. Gesell Developmental Schedule was used to assess the development quotient (DQ) of the infants between 18 to 36 months. The intelligence quotients (IQ) of the preschool children between 48 to 72 months were tested with WPPSI.

Results: Either in neonatal or in infant period, no significant difference was noted between the asymptomatic congenital CMV infection children and the controls in average weight, height and head circumference (both p >0.05). The intellectual development was disproportion in asymptomatic congenital infected children. Compared with the control group, both global DQ and full-scale IQ scores of asymptotically infected children were worse (t=2.19, p =0.031; t=2.48, p =0.015), especially on language DQ scores (t =3.25, p =0.002) and verbal IQ scores (t =3.88, p =0.000). However, the incidence rates of mental retardation (DQ/IQ <70) were similar in two groups (χ² =1.03, p >0.05).

Conclusions: Although asymptomatic congenital CMV infection did not have significant influence on the neonatal physical development or incidence of mental retardation later in life, it is obviously an important factor correlating with long-time cognitive outcomes, especially on the development of language. It is necessary to survey CMV congenital infection and monitor the early intellectual development of children with asymptomatic congenital CMV infection in this area.
Prenatal Diagnosis, Prognostic Indicators, and Treatment

P-38 Evaluation of the VIDAS, LIAISON and AxSYM CMV IgG & IgM Reagents on Routine and Interfering Samples.

Background: CMV serology interpretation encounters many pitfalls because of IgM positive results due to various causes (interference, cross-reactions and non specific immune stimulation).

Objective: The objective of the study was to evaluate the performances of CMV IgG and CMV IgM tests on the following systems: VIDAS (bioMérieux SA, France), AXSYM (Abbott, USA) and LIAISON (DiaSorin, Italy).

Methods: The following clinical samples were tested with the 3 above mentioned systems except for the 20 samples with low IgG levels: 200 pregnant women serum samples collected from the laboratory routine 20 serum samples with potential interferences 20 serum samples from CMV primary infection 20 serum samples with low CMV-specific IgG levels according to Liaison or VIDAS methods.

Results: 1- CMV IgG assays: 200 routine samples were tested and 2 gave discrepant results with AxSYM compared to Liaison or VIDAS. When testing 20 potentially interfering samples, rheumatoid factor samples yielded a true discrepant result, positive with AxSYM and negative with VIDAS and Liaison. 2- CMV IgM assays: After resolution of the discrepancies among the 200 serum samples tested, 181 were found negative and 4 positive with the 3 systems. 15 serum samples gave discrepant results between techniques and AxSYM presented more IgM false positive results than the 2 other methods. Concerning samples with potential interferences and EBV infection, half, if not all of them, yielded discrepancies between techniques.

Conclusion: For CMV IgG, a very good concordance was found between the 3 systems studied with all kind of samples. CMV IgM study was more challenging. Concerning sensitivity estimated from primary infections samples: Liaison detected all of them, VIDAS detected all, but one, and AxSym 2 out of 6, only. For specificity, VIDAS presented the best performance on both routine and potentially interfering samples. AxSym was shown to be cross-reactive with all primary EBV infection samples and with some auto-antibodies samples, while Liaison gave a few false positive results with the latter kind of interfering samples.

P-39 Evaluation of Architect and Elecsys CMV IgM Assays, by Public Health Laboratory in Israel.
Yoav Dickstein, Irena Lipkin, Simona Takacs. Leumit Health Fund, Petach Tiqva, Israel.

Background: CMV IgG and IgM tests of choice to screen and diagnose pregnant women by a public health care laboratory serving 700,000 health insured patients, representing 10% of the population in Israel (high prevalence of EBV infections), depends on the sensitivity and specificity measured in the population being served.

Objectives: To report clinical evaluation results of Architect® and Elecsys® CMV IgM.

Methods: A)512 unselected routine serum samples ordered for anti CMV tests (428 women, 56 % pregnant) were collected, centrifuged, stored overnight at 4°C, and assayed the next day. B)59 serum samples (31 pregnant women) with primary CMV infections. C)20 serum samples from primary EBV infection patients, and D)20 serum samples from patients with autoantibodies were thawed and tested on the same day. Sera were tested using Architect® (ABBOTT Diagnostic Division) and Elecsys® (ROCHE Diagnostics) CMV IgM and IgG, systems and kits. Samples, which resulted in CMV IgM discrepant results, were tested for CMV IgG using CMV IgG AVIDITY EIA WELL (RADIM S.p.A).

Results: 1% of unselected routine samples were confirmed having asymptomatic primary CMV infection. Architect® and Elecsys® CMV IgM specificities were 92.7 and 97.2 respectively. Sensitivities were 100.0% and 93.2% respectively. Of 59 CMV primary infection samples 3 (5.1%) were equivocal using Architect® and 9 (15.2%) were equivocal using Elecsys®. Four first drawn sample from 31 primary CMV infection patients, were positive by Architect®, and negative by Elecsys®. Of 20 EBV primary infection samples, 11 were positive by Architect®, and 4 by Elecsys® CMV IgM. Of 20 samples having autoantibodies, 19 were negative by Architect®, and 20 by Elecsys®.

Conclusions: Primary EBV infection is the major cause for false positive CMV IgM results. CMV IgM equivocal or positive results, coupled with low to moderate avidity results, should be suspected for primary CMV infection. Both methods compared are suitable provided specific peculiarities of each are considered. For the purpose of screening pregnant women for primary CMV infection during the first trimester, a high sensitivity assay is the choice for a public health laboratory serving 10% of the Israeli population in a country with high prevalence of EBV infection.
**P-40 Evaluation of the Abbott ARCHITECT CMV Panel in Pregnant Women with Primary CMV Infection.**
Gregory Maine, Rene Stricker, Reto Stricker. Abbott Diagnostics, Abbott Park, IL.

**Background:** What serologic tests should be used to screen pregnant women for CMV infection.

**Objectives:** Evaluate the performance of the ARCHITECT CMV IgM, IgG, and IgG avidity assays in women with documented seroconversion for CMV during gestation.

**Methods:** The three CMV assays for the ARCHITECT instrument are two-step immunoassays utilizing CMV virus lysate coated paramagnetic microparticles for the capture of human anti-CMV antibodies. The CMV IgM assay also contains both viral lysate and the recombinant protein CKS-pp150, pp52 (UL32, UL44) coated onto paramagnetic particles. Samples (n = 136) from 31 pregnant women with documented recent seroconversion were tested on the Abbott ARCHITECT CMV assays and the results were compared to the Abbott AxSYM CMV IgG, AxSYM or IMx CMV IgM assays, and a modified Dade Behring CMV IgG avidity assay.

**Results:** The seroconversion sensitivity of the ARCHITECT CMV IgM assay was approximately equivalent to the AxSYM and IMx CMV IgM assays with the ARCHITECT assay detecting the same bleed as positive relative to IMx (n = 19) and one bleed earlier than AxSYM (n = 13). The seroconversion sensitivity of the ARCHITECT CMV IgG assay was slightly greater than the AxSYM CMV IgG assay as shown by detection of the first bleed as CMV IgG positive by ARCHITECT in 2/31 patients before AxSYM. Detection of CMV-specific IgM before CMV IgG also occurred in 6/31 patients (19%) with a window period of approximately 8-13 days. The clinical sensitivity of the ARCHITECT CMV IgG avidity assay using a cutoff of 4 months post-seronegative bleed was (62/64) = 96.9% (95% CI = 89.2-99.6%). The relative agreement between the ARCHITECT and modified Dade CMV IgG avidity assays was (71/80) = 88.8% (95% CI = 79.7-94.7%).

**Conclusion:** The performance of the three fully automated Abbott ARCHITECT CMV immunoassays, including the reflex CMV IgG avidity assay, was equivalent to the reference assays. The observation that detection of CMV-specific IgM before IgG occurred in 19% of the patients tested emphasizes the importance of testing pregnant women with both a sensitive CMV IgM and IgG test at the same time to avoid false negative results during early seroconversion.

**P-41 CMV Replication in the Human Placenta: Prospects for Early Diagnosis Using Chorionic Villus Samples.**
Susan McDonagh, Naoki Nozawa, Mary Norton, Lenore Pereira. Department of Cell and Tissue Biology, University of California San Francisco, CA.

**Background:** Pregnant women with primary CMV infection transmit virus to the fetus (40%) and symptomatic congenital disease occurs in 25% resulting in permanent birth defects. Our laboratory reported that CMV spreads from the infected uterine vasculature to invasive cytotrophoblasts and disseminates to the adjacent placenta as immune complexes that are transcytosed across syncytiotrophoblasts and sequestered in caveolar vesicles. Low to moderate IgG avidity to CMV is associated with infection in villous cytotrophoblasts that spreads to stromal fibroblasts and fetal blood vessels in the villous core. Accordingly, 63% of infected placentas contain CMV DNA and virion glycoprotein B, and subset also contained viral replication proteins.

**Objectives:** Determine whether specimens from chorionic villus sampling (CVS) contain viral DNA and RNA sufficient for diagnosis of active infection.

**Methods:** Excess villi from CVS were obtained from women attending the UCSF Center for Reproductive Medicine for genetic testing between 10 and 12 weeks gestation because of maternal age or abnormal nuchal translucency scan. To assess active replication, CMV DNA and RNA were extracted and analyzed by conventional PCR using primer sets for immediate early (IE) and gB, and by RT-PCR for IE and cmvIL-10 transcripts, respectively.

**Results:** Of 100 CVS tested, 50 (55%) contained CMV DNA. In 78 specimens sufficiently large to assess viral RNA, 5 (6%) contained viral gene transcripts.

**Conclusion:** CVS could be used for supplementary early diagnostic test at 10-12 weeks of gestation in samples obtained for prenatal diagnosis of fetal chromosomal, biochemical and molecular disorders. In addition to prenatal ultrasound findings and assessment of maternal immunity, detection of CMV replication in CVS could enable early diagnosis of potential congenital infection in concert with low-avidity maternal IgG. Improved prenatal diagnosis of congenital CMV infection in first trimester of gestation could spur development of novel treatments to prevent congenital disease.
P-42 Comparative Evaluation of 8 Commercial Kits for IgG Avidity Determination to Human Cytomegalovirus.
Maria Grazia Revello, Emilia Genini, Giovanna Gorini, Giuseppe Gerna. Servizio di Virologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

Background: Currently, human cytomegalovirus (HCMV) screening of pregnant women is not recommended. However, in many European countries, a fair proportion of pregnant women is routinely tested for IgG and IgM to HCMV and, whenever a positive IgM result is obtained, an IgG avidity testing is usually performed. Indeed, based on the availability of the avidity assay, some companies have developed highly sensitive IgM assays, claiming that IgG avidity testing would have allowed a correct interpretation of positive IgM results.

Objectives. Aim of the present study was to evaluate and compare performances of the eight HCMV IgG avidity assays currently available in Europe.

Materials and Methods: The following groups of sera were tested: i) 199 sequential samples collected from 67 pregnant women 7-275 (median 75) days from the onset of primary HCMV infection documented by IgG seroconversion; ii) 49 sequential sera from 39 pregnant women referred to our Institution at ≤12 weeks' gestation because of a positive IgM result; iii) 6 sera samples of a HCMV IgG, IgM and IgG avidity proficiency panel developed by Gisela Enders (Stuttgart, Germany) for the European Congenital CMV Initiative. All 254 samples were tested by each of the eight (Abbott, BioMérieux, DiaSorin, Bio-Rad, Diesse, Euroimmun, Radim, and Technogenetics) HCMV IgG avidity assays commercially available according to manufacturers’ instructions.

Results: Preliminary results indicate that in group i), the number of sera containing low, moderate or high avidity IgG according to the eight kits tested varied from 80 to 181, 32 to 156, and 15 to 66, respectively. Not a single serum sample out of the 199 tested was scored identically by kits under evaluation. In the group ii) concordant results were obtained in only 2/39 (5.1%) women, whereas in group iii) only the IgG-negative serum was correctly identified by all kits. Therefore, overall concordance was exceedingly low (3/254, 1.2%).

Conclusions: HCMV IgG avidity assays need to be improved and standardized before they can be reliably used for IgM interpretation on a routine basis. As a corollary, at the moment, highly specific IgM assays should be preferred for screening purposes in pregnant women.

Public Awareness and Behavioral Interventions

P-43 Awareness of human cytomegalovirus (HCMV) infection among pregnant women in Italy.
Maria Barbi, Agata Calvario, Tiziana Lazzarotto, Enrico Ferrazzi, Marcello Lanari, Brunella Guerra, Irene Cetin, Annamaria Marconi, Guiseppe Loverro, Antonella Vimercati, Allessia Arossa, Elisa Fabbri, Maria Grazia Revello. Dipartimento SaMiVi, Università degli Studi di Milano, Milano, Italy.

Background: Knowledge that human cytomegalovirus (HCMV) is the leading cause of congenital infection and that the risk of HCMV infection during pregnancy can be reduced by simple hygienic measures does not seem to be widespread among Italian pregnant women. HCMV serologic testing in pregnancy, besides assessing maternal serostatus, should be an important opportunity for obstetricians for providing seronegative women with sound information about HCMV infection and its preventive measures. Although this testing in pregnancy is not recommended in Italy, a fair number of pregnant women seems to be routinely tested. Therefore, we started a survey to verify both the actual rate of HCMV testing and the level of awareness of HCMV infection among pregnant women.

Objectives: To evaluate: i) the rate of HCMV testing in pregnancy and ii) the effect of testing on awareness of HCMV infection among women of child-bearing age.

Materials and Methods: A short (four questions) self-administered written questionnaire is currently being proposed to women who delivered in seven hospitals (about 14500 deliveries per year altogether) of three Italian regions. The four questions are: 1) if and when was the testing performed; 2) which was the result; 3) if the test was repeated, and 4) whether the woman, if seronegative, was given instructions to prevent the infection. Responses will be evaluated against number of children, age and instruction level.

Results: Preliminary data from four hospitals indicate a good compliance and a satisfactory comprehension of the questions. This makes us confident that we will be able to present extended results at the Atlanta Conference.

Conclusions: Data from this study will be important in assessing: i) the actual rate of HCMV testing in pregnancy in representative Italian regions, and ii) whether obstetricians are acting as health educators. In addition, groups (women, healthcare providers) needing interventions for raising awareness of HCMV infection, will be identified.
**Vaccines**

**P-44  BAC cloning and cell tropisms of viruses derived from the Towne vaccine.**  
Michael A. McVoy, Xiaohong Cui.  Department of Pediatrics, Virginia Commonwealth University School of Medicine, Richmond, VA.

**Background:** The cytomegalovirus live attenuated Towne vaccine is an equimolar mix of two variants, Towne-short and Towne-long. Towne-short is missing ~15 kb of sequence relative to Towne-long, but neither genome has been fully sequenced. Endothelial tropism of these viruses is important as infection of endothelial cells may promote vascular disease. Towne-short is non-endothelial tropic because a mutation in UL130 renders the UL130 protein poorly expressed and this prevents formation of a gH/gL/UL128/UL130/UL131 virion complex that is essential for endocytic entry into endothelial and epithelial cells. However, repair of UL130 is associated with acquisition of endothelial tropism by Towne-short upon in vitro passage in endothelial cells. This suggests a potential for Towne viruses to become endothelial tropic in vivo.

**Objectives:** To derive from the vaccine bacterial artificial chromosome (BAC) clones of Towne-short and Towne-long genomes and characterize the tropisms of both viruses.

**Methods & Results:** BACs TS15 and TL12 were derived and shown by restriction pattern and sequencing to accurately represent Towne-short and Towne-long genomes with the exception of ~7-kb deletions to the right of the BAC origin that remove US29-TRS1. Transfection of TS15 or TL12 into MRC-5 fibroblasts reconstituted viruses that replicated in MCR-5 cells with efficiencies and kinetics identical to the parental viral mixture. Neither virus was able to infect ARPE-19 human retinal epithelial cells, suggesting that both Towne-short and Towne-long are defective for endocytic entry. However, transfection of ARPE-19s with BAC TS15 and long-term culture (>30 days) reconstituted a virus designated TS15epi that replicated with high efficiency in ARPE-19s. Surprisingly, UL130 was not repaired in TS15epi.

**Conclusions:** That TS15epi retains the UL130 mutation suggests that epithelial tropism is conferred by a compensatory mutation elsewhere in the genome. Identifying the mutation(s) and understanding how epithelial tropism occurs in a UL130 mutant background may reveal novel aspects of viral entry. Mutations that disrupt the gH/gL/UL128/UL130/UL131 complex may limit a vaccine strain's ability to induce antibodies that neutralize endocytic entry. This may explain why Towne is deficient in this regard. Mutations that permanently disable endothelial tropism without disrupting the gH/gL/UL128/UL130/UL131 complex would be highly desirable for future live vaccine strains.

**P-45  CMV vaccines fail to induce epithelial entry neutralizing antibodies comparable to natural infection.**  
Michael A. McVoy, Xiaohong Cui, Benjamin P. Meza, Stuart P. Adler.  Department of Pediatrics, Virginia Commonwealth University School of Medicine, Richmond, VA.

**Background:** Antibodies that neutralize cytomegalovirus (CMV) entry into fibroblasts are predominantly directed against epitopes within virion glycoproteins that are required for attachment and entry. However, because the mechanism of CMV entry into epithelial and endothelial cells differs from fibroblast entry, antibodies that neutralize epithelial cell entry may differ from those that neutralize fibroblast entry.

**Objectives:** To determine if neutralizing titers differ significantly when epithelial cells are used as targets, as compared to fibroblasts, and to compare Towne and gB/MF59 vaccines to natural infection with regard to induction of epithelial entry neutralizing antibodies.

**Methods:** A GFP-based assay was developed to accurately measure in parallel serum neutralizing activities against CMV entry into MRC-5 fibroblasts and ARPE-19 epithelial cells.

**Results:** Human immune sera and CMV-hyperimmunoglobulins had on average 48-fold higher neutralizing activities against epithelial cell entry compared to fibroblast entry. Despite gB ELISA titers that were comparable to (Towne) or 8-fold higher than (gB/MF59) natural infection sera, the Towne vaccine and the gB/MF59 subunit vaccine induced epithelial entry neutralizing activities that were on average 25-fold (Towne) or 13-fold (gB/MF59) lower than those observed following natural infection. Adsorption with recombinant gB was effective in removing epithelial neutralizing activity from a gB/MF59 vaccine recipient serum but had no measurable effect on a natural infection serum.

**Conclusions:** Our results confirm those reported by Gerna et al. (J. Gen. Virol. 2008;89:853-65) suggesting that natural CMV infections induce a previously unrecognized epithelial entry-specific neutralizing activity. Our data further suggest that this activity is substantial; on average human immune sera were 48 times more potent in blocking
epithelial cell entry than fibroblast entry. Importantly, neither vaccine was able to induce epithelial neutralizing titers comparable to natural infection. Although gB/MF59 did elicit some epithelial neutralizing activity, both adsorption studies and gB ELISA titers suggest that epitopes recognized by antibodies comprising the epithelial neutralizing activity in natural immune sera do not lie within gB. These results suggest that CMV vaccine efficacy may be enhanced by strategies designed to induce greater levels of epithelial entry-specific neutralizing antibodies, if possible equivalent or superior to activities induced by natural infection.

**P-46 Novel CMV pDNA Vaccines.**
Ronald Moss. Vical Incorporated, San Diego, CA.

The use of plasmid DNA (pDNA) vaccines to prevent CMV infections represents a novel strategy that has advantages over conventional vaccine approaches. Multiple CMV antigens can be included in the same pDNA vaccine in order to stimulate both humoral and T-cell immunity. Historically, pDNA vaccines have had good safety profiles. Because they are non-viral, pDNA vaccines are not limited by anti-vector immunity, and can be administered repeatedly. In addition, pDNA vaccines can be manufactured rapidly on a large GMP scale and are stable for extended periods. Both unformulated and formulated CMV pDNA vaccines have been tested in humans with encouraging results. In a previous clinical trial, an unformulated CMV pDNA vaccine induced CMV-specific T-cell (P<0.05) and B-cell (P<0.05) priming as shown by the induction of anamnestic responses following administration of live, attenuated CMV Towne strain in healthy volunteers. In a Phase 1 clinical trial of CMV bivalent (gB and pp65) pDNA vaccine formulated in CRL1005 poloxamer, a non-ionic block copolymer, IFN-γ T-cell immune responses were detected by cultured ELISPOT assay in approximately 70% of healthy volunteers. pDNA vaccines formulated with Vaxfectin®, a cationic lipid-based adjuvant, have induced potent antibody responses in animal models of influenza, measles, and dengue and resulted in protection from infection after viral challenge. Preliminary results of Vaxfectin®-formulated CMV plasmids in animals suggest robust CMV-specific humoral immune responses. Vaxfectin®-formulated CMV pDNA vaccine may be an ideal product development candidate for women of childbearing potential to prevent congenital CMV infections.
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Planning Begins now!

September 23-24

International Congenital Cytomegalovirus Conference

For more information, contact:

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