Oral Presentation Abstracts

Section: Viral structure, Entry, Replication and Cell Biology

Title: Amino acid residues that influence activation and/or activity of the paramyxovirus fusion (F) glycoprotein

Authors: Joanna Rawling, Concepción Palomo, Blanca García-Barreno and José A. Melero

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Abstract: We are currently investigating two aspects of the paramyxovirus fusion (F): i) the relevance of proteolytic cleavage for protein activation and ii) the significance of specific amino acid changes for resistance to peptides that correspond to heptad repeat B sequences.
On the one hand, whereas most paramyxovirus F proteins are cleaved only once during maturation, the respiratory syncytial virus (RSV) F protein is cleaved twice, at two furin sites separated by 27 amino acids. In addition, most paramyxovirus F proteins require the cooperation of the receptor binding protein (HN or H) for membrane fusion; however, RSV F is independent of the attachment (G) glycoprotein for fusion activity. To investigate whether or not these two differences are interconnected, a series of mutations were introduced in a cDNA copy of the Sendai virus (SeV) F gene to reproduce partially or completely the RSV cleavage sites and the intervening sequence between them. The mutants were subsequently tested in two different cell-cell fusion assays. The results obtained indicate that the double cleavage of RSV F (and loss of the intervening peptide) somehow substitutes for the participation of the receptor binding protein in controlling the activation and activity of the SeV F protein.
On the other hand, we have confirmed that peptides of a certain length, which reproduce sequences of the RSV F protein heptad repeat B (HRB), neutralize virus infectivity. To investigate their mechanism of action, RSV escape mutants were isolated after repeated passage of the virus in the presence of HRB peptides. Ten independent resistant viruses were selected containing single amino acid substitutions that changed four different residues of the RSV F protein. None of the changes occurred in HRB. However, in a 3-D model of RSV F, these residues were located near amino acid changes reported for mutants resistant to small synthetic drugs. Interestingly, some of the peptide escape mutants have lost the capacity to form large syncytia. In addition, the amino acid change in one of these mutants is identical to that described in a virus resistant to a neutralizing antibody. Hence, the peptide escape mutants identify residues that influence the fusogenic capacity of RSV F without ablating its activity.

 # 1 Section: Viral Structure, Entry, Replication and Cell Biology

Title: Role of Human Respiratory Syncytial Virus glycoproteins in apical targeting during viral maturation in polarized epithelial cells.

Authors: Melissa Batonick[1], Antonius G.P. Oomens[1], Gail W. Wertz[1]

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Abstract: Human Respiratory Syncytial Virus (HRSV), an enveloped negative sense single-stranded RNA virus, encodes eleven known gene products. Of these, three are viral transmembrane glycoproteins, the small hydrophobic protein, SH, the attachment protein, G, and the fusion protein, F.

During the course of infection of both polarized epithelial cell lines and ciliated human airway epithelial cells, the virus matures and buds from the apical surface. The viral gene products responsible for this directional release are largely unexplored, although one previous study, using overexpressed fusion glycoprotein in the absence of other viral proteins, implicated the transmembrane domain of F as containing intrinsic apical targeting signals.

With some viruses, such as VSV, Measles, and Marburg, directional targeting is affected by multiple viral proteins. Therefore, we chose to examine the contribution of each of the HRSV glycoproteins to apical targeting and release, in the context of infectious virus. We generated HRSV viruses engineered to have the three glycoprotein genes deleted either individually or in groups. These viruses were used to infect polarized epithelial cells, grown on porous substrates. Prior to viral budding, HRSV proteins traffic from the site of synthesis to the site of release at the apical membrane. To study the impact of each glycoprotein on viral protein sorting to the apical plasma membrane, the cell surface localization of F and/or G glycoproteins as well as the intracellular localization of the nucleocapsid N protein were monitored by confocal microscopy. In conjunction, a cell-based ELISA quantified the amount of transmembrane glycoproteins that reached the apical and basolateral cell surfaces after infection. We also analyzed the site of directional release of the engineered viruses using an anti-N ELISA on the apical and basolateral supernatants. Our data suggest that the three viral glycoproteins, individually or in combination, are not the major determinants of apical sorting or release of HRSV when in the presence of all other viral proteins.

2 Section: *Viral Structure, Entry, Replication and Cell Biology*

Title: Persistent of Respiratory Syncytial Virus In Human Dendritic Cells and Influence of Nitric Oxide

Authors: Lynsey Hobson and Mark L Everard

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Abstract: The annual epidemics of respiratory syncytial virus (RSV) infection are probably explained by poor herd immunity and the existence of a dormant reservoir of virus that is activated by an unknown trigger. The virus causes particular problems in infants, the elderly and patients with COPD.

During two consecutive winters, human monocyte derived dendritic cells [DCs] were exposed on a single occasion to one of two forms of RSV labeled with a fluorescent expresser genes [rgRSV or rrRSV] during the epidemic season. The cultures were maintained for many months with fresh DCs being added at monthly intervals. The cultures were variously exposed to 600ppb nitric oxide for 15 minutes, nitric oxide [NO] donors and NO inhibitors outside of the RSV epidemic season. The pattern of productive infection of DCs *in vitro* appeared to parallel the natural epidemics in that DCs only exhibited evidence of viral replication and productive infection as manifest by intra cellular fluorescence and infection of HeLa cells during the RSV epidemic season. When the long term cultures were exposed to the above agents outside of the RSV epidemic season there was again evidence of vigorous replication and productive infection as evidenced by the reappearance of fluorescence and productive infection of HeLa cells.

The results indicate that RSV may remain dormant in dendritic cells for prolonged periods and that replication appears to be activated by suppression of endogenous NO production. These observations may be key to our understanding of the mechanisms contributing to the annual epidemics of RSV infection.

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 # 3 Section: Viral Structure, Entry, Replication and Cell Biology

Title: Mechanisms of RNA Synthesis Initiation in Respiratory Syncytial Virus

Authors: Vanessa M. Cowton[1], David R. McGivern[1], Sarah L. Noton[2] and Rachel Fearns[1,2]

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Abstract: Respiratory syncytial virus (RSV) undergoes two different types of RNA synthesis initiation: 3´ initiation, which occurs in response to initiation signals at the 3´ termini of genome and antigenome RNAs, and gene start (GS) initiation, which occurs internally on the genome template. In this study, we investigated what effect substituting the first nucleotide (nt) of an initiation signal in the template would have on the sequence of the RNA product. To examine the 3´ initiation event, a template that signals highly efficient RNA replication was created by constructing a minigenome with a replication optimal promoter at its 3´ end. The first nt of this replication template was mutated from a U to an A residue, and the antigenome produced was quantitated by Northern blot analysis and its sequence determined by 5´ RACE. This analysis showed that the 1 U-to-A substitution resulted in antigenome synthesis at ~40% of the parental template level, indicating that the mutant template maintained a significant level of 3´ initiations. Sequence analysis of the population of RNAs produced from this template showed that almost all contained a non-templated, but wild-type, A residue at the first position, rather than a templated U residue. This was the case even if the minigenome was limited to a single step of RNA replication so that selection of an optimal sequence over multiple cycles of replication was prevented. A previous study had suggested that mRNA initiation at the RSV GS signal might also involve non-templated initiation, however, examination of initiation products from a mutant GS signal indicated that the majority of GS initiations are template directed, albeit with a relatively high error rate. These data suggest that there are fundamental differences between the mechanisms of 3´ and GS mediated initiation. Selection of the 3´ initiating nt in a template independent manner might provide a mechanism by which the virus maintains the integrity of its genome termini.

 # 4 Section: Viral Structure, Entry, Replication and Cell Biology

Title: Structures of the pre- and post-entry paramyxovirus F protein: implications for RSV vaccine and therapeutic development

Authors: Xiaolin Wen[1], Ping Yuan[1], Hsien Shen Yin[2], Sarah Connolly[3,4], Robert A. Lamb[3,4] and Theodore Jardetzky[1]

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Abstract: The paramyxoviridae are enveloped viruses that include, among others, respiratory syncytial virus (RSV), parainfluenza viruses, mumps virus and measles virus. Like other enveloped viruses, the paramyxoviruses require fusion of the viral and cellular membranes to enter a host cell. The fusion (F) protein catalyzes this membrane merger step by initially folding to a metastable conformation and subsequently refolding during this process.

We determined the crystal structures of two paramyxoirus F proteins, in the pre- and post-fusion conformations. The structure of the secreted hPIV3 F ectodomain folds to the post-fusion conformation, exhibiting a prototypical 6-helix bundle whose formation is tightly linked with membrane fusion. For the PIV5 F protein, we determined the structure of the pre-fusion conformation, after stabilizing the metastable state by the addition of a C-terminal trimerization domain. The comparison of the pre- and post-entry F structures reveals major conformational differences, involving transformations in secondary and tertiary structure. Models of the RSV F protein have also been generated and biochemical evidence indicates that RSV F undergoes similar conformational changes, with important implications for RSV vaccine and therapeutic development.

 # 5 Section: Immunology – Innate and Adaptive

Title: Macrophage Insufficiency Underlies Type I Interferon Mediated Apoptotic Crisis in Respiratory Syncytial Virus Bronchiolitis

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Abstract:

Although respiratory syncytial virus (RSV) infection is the most important cause of bronchiolitis in infants, the pathogenesis of RSV disease is poorly described. We evaluated histopathologic features of bronchiolitis in lung tissues from fatal cases of human RSV infection. Airways occlusion with cellular debris and serum protein was consistently observed, along with apoptotic respiratory epithelium and dense inflammatory infiltrate. Similar features were found following RSV infection in New Zealand Black mice but not MHC matched BALB/c mice. Macrophage uptake of infected and apoptotic cells was markedly attenuated in NZB mice. Deficient alveolar macrophages appear central to enhanced disease, as alveolar macrophage depletion in BALB/c mice prior to RSV exposure resulted in airways occlusion and dense inflammatory infiltrate, similar to those observed in NZB mice. In macrophage insufficient mice, RSV infection yielded increased viral load and epithelial expression of type I interferon induced genes. Antibody blockade of the type I interferon receptor during RSV infection in NZB mice increased viral load, but reduced lung inflammation and airways occlusion, particularly in combination with an RSV-F neutralizing antibody. Together our data revise current concepts of RSV immunity and pathogenesis, suggesting that innate rather than adaptive immune responses are critical determinants of RSV bronchiolitis severity.

#6 Section: Immunology-Innate and Adaptive

Title: Genetic susceptibility to Respiratory Syncytial Virus bronchiolitis is predominantly associated with innate immune genes.

Authors: Riny Janssen[1], Louis Bont[2], Christine L.E. Siezen[1], Hennie M. Hodemaekers[1], Marieke J. Ermers[2], Gerda Doornbos[3], Ruben van 't Slot[4], Ciska Wijmenga[4], Jelle J. Goeman[5], Jan L.L. Kimpen[2], Hans C. van Houwelingen[3, 5], Tjeerd G. Kimman[6] and Barbara Hoebee[1].

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Abstract: Respiratory syncytial virus (RSV) is a common cause of severe lower respiratory infection in infants. Only a proportion of infected children require hospitalization, and known risk factors for severe disease, like premature birth, cannot fully explain differences in disease severity. Genetic factors have been implicated in this process. To study the complexity of RSV susceptibility, and to identify genes and biological pathways involved, we performed a genetic association study in 470 children hospitalized for RSV bronchiolitis, their parents and 1008 controls, and analyzed 384 SNPs in 220 candidate genes that play a role in airway mucosal responses, innate immunity, chemotaxis, adaptive immunity, and allergic asthma. SNPs in innate immune genes, i.e. *VDR* (rs10735810), *JUN* (rs11688), *IFNA5* (rs10757212), *NOS2* (rs1060826), displayed the strongest association with disease severity which was reinforced by the association of RSV bronchiolitis with innate immunity as a process, when applying the global test for groups of genes.

 # 7 Section: Immunology – Innate and Adaptive

Title: Restoration of effector activity in memory $CD8^+$ T cells following infection with a recominant Respiratory Syncytial Virus lacking the transmembrane glycoproteins.

Authors: Jonathan P. Castillo[1], Tom Oomens[2], Gail W. Wertz[2,3], Thomas J. Braciale[1,2,3]

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Abstract: Respiratory syncytial virus (RSV) is one of the most common causes of viral bronchiolitis and a major cause for hospitalization in infants and the elderly. One notable feature of RSV is that individuals naturally infected with the virus are susceptible to re-infection with RSV. This observation suggests that RSV has evolved a mechanism to modulate the host adaptive immune response. Using a murine model to examine the immune response to RSV *in vivo*, our laboratory has previously shown that a significant fraction of virus-specific $CD8^+$ T cells in the lungs exhibit an impaired capacity to produce effector cytokines and degranulate in response to peptide stimulation. Additionally, we demonstrated that the observed defect in $CD8^+$ effector activity is associated with the expression of a RSV gene product in the lungs. To address whether the expression of the RSV transmembrane glycoproteins contributes to the observed defect in effector T cell activity, we examined the $CD8^+$ effector T cell response to infection with a recombinant RSV mutant that lacks the open reading frames encoding the SH, G, and F glycoproteins (RcRSVΔSH-G-F). We find that the RcRSVΔSH-G-F mutant can induce inflammation as measured by the recruitment of $CD45^+$ cells as well as $CD4^+$ and $CD8^+$ T cells to the lungs following infection. In comparison to WT RSV, the RcRSVΔSH-G-F mutant induces a similar frequency of $M2_{82\text{-}90}$-specific $CD8^+$ T cells in the lungs in both the primary and memory responses to infection. Notably, we find that infection with the RcRSVΔSH-G-F mutant induces a defective primary $CD8^+$ effector T cell response that is comparable to that observed in the WT RSV-infected lungs. In contrast, we find that in the recall response to RcRSVΔSH-G-F infection, the $M2_{82\text{-}90}$-specific $CD8^+$ T cells in the lungs no longer express a defective effector response as seen with WT RSV infection. Furthermore, we find that the quality of the effector response is enhanced following RcRSVΔSH-G-F infection. Taken together, our results indicate that the RSV glycoproteins do not account for the defect in the $CD8^+$ effector T cells responding to primary virus infection in the lungs but their absence leads to an improved recall $CD8^+$ T cell response to infection.

8 Section: Immunology – Innate and Adaptive

Title: **A central role for neonatal CD8 cells in the inhibition of antibody boosting and disease enhancement during adult re-infection**

Authors: John S Tregoning, Yuko Yamaguchi, James Harker, Belinda Wang, Debbie Lee and Peter JM Openshaw.

Affiliation: Department of Respiratory Medicine, MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, National Heart and Lung Institute, Imperial College London, St. Mary's Campus, London W2 1PG, UK.

Abstract: Neonates are highly susceptible to many infections and respond poorly to vaccines. In neonatal mice, the timing of primary infection RSV determines responses to later re-infection. Compared to adults, neonatal BALB/c mice developed only mild disease and recruited CD8 cells that were defective in interferon gamma production after RSV infection. Neonatally infected mice developed poor anti-RSV antibody responses, allowing re-infection in adulthood; such secondary infections caused enhanced inflammation and profuse lung T cell recruitment, but boosted anti-RSV antibody titers. CD4 cell depletion during secondary RSV challenge attenuated disease and inhibited antibody boosting. Depletion of CD8 cells also markedly attenuated disease severity but enhanced lung eosinophillia. Remarkably, transient CD8 cell depletion during primary neonatal infection reduced disease and enhanced antibody boosting during secondary challenge during adulthood. Therefore, CD8 cells play a central role in the outcome of neonatal infection, enhancing disease during secondary challenge and profoundly affecting antibody responses during re-infection in adulthood. These findings demonstrate a crucial role for CD8 T cells in the regulation of immune responses after neonatal infection and have important implications for early-life vaccine strategies.

 # 9 Section: Immunology – Innate and Adaptive

Title: Analysis of the specificity of bovine $CD8^+$ T cells primed by BRSV and identification of a novel BRSV-specific $CD8^+CD4^+$ T cell subset

Authors: Geraldine Taylor[1], Sara Wyld[1], Birgitte Viuff[2], Shirley Ellis[1] and Julie Furze[1]

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Abstract: $CD8^+$ T cells play a major role in the clearance of BRSV from the bovine respiratory tract. We have investigated the BRSV proteins recognised by purified $CD8^+$ T cells, from 9 BRSV-immunised cattle, following restimulation, *in vitro*, with BRSV-infected, MHC-matched macrophages. These cultured $CD8^+$ T cells were screened in an Elispot assay for IFNγ production in response to rFPV expressing 8 of the 11 BRSV proteins. The P protein was the only BRSV protein recognised by BoLA A31 and A20 cattle and was the predominant protein recognised by A18 cattle. BoLA A18 cattle also recognised the M, N and G proteins. BoLA A14 cattle recognised the G, F, M and M2 proteins; and BoLA A10 cattle recognised the G, M, N and NS1 proteins. MHC class I restriction of IFNγ production was demonstrated using BRSV-infected P815 cells transfected with different bovine MHC class I genes. Furthermore, $CD8^+$ T cells restimulated *in vitro* with rFPV-P infected macrophages showed MHC-restricted lysis of BRSV-infected skin fibroblasts. Using overlapping peptides from the BRSV P protein a BoLA A18-restricted 9-mer peptide corresponding to amino acids 141 to 149 (KLSEIIGML) was identified. This is the first BRSV epitope recognised by bovine $CD8^+$ T cells to be defined.

Following restimulation with virus-infected macrophages, a proportion of $CD8^+$ T cells were identified that also expressed CD4. The proportion of $CD8^+CD4^+$ T cells increased with successive restimulation, *in vitro*. These double positive cells were BRSV protein-specific and $CD3^+$, CD8α/β^+, $NKp46^-$, γ/δ TCR^-. A similar subset of extrathymic $CD8^+CD4^+$ T cells has been demonstrated in humans and mice, where it has been shown that CD4 on $CD8^+$ T cells can enhance effector function and can function as a chemotactic receptor allowing cells to migrate in response to IL-16, which is produced by T cells, mast cells, eosinophils and bronchial epithelial cells. Further studies are required to determine the role of these cells in BRSV infection, *in vivo*.

#10 Section: Immunology – Innate and Adaptive

Title: Decreased Expression of Class II MHC on Dendritic Cells in Infants with RSV

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Abstract: Viruses utilize multiple strategies to evade detection by the immune system, including downregulation of major histocompatability complex (MHC) proteins on antigen presenting cells. We investigated the MHC-Class II surface expression on dendritic cells in children with acute RSV infection. Nasopharyngeal samples (NPS) and blood were collected from twenty patients hospitalized with acute RSV bronchiolitis and 13 healthy control children. Myeloid dendritic cells (mDCs) were identified in NPS and blood samples by flow cytometry as $CD3^-$,$CD14^-$, $CD16^-$, $CD19^-$, $CD20^-$, $CD56^-$, HLA-DR^+, $CD11c^+$ cells. The median fluorescent intensity (MFI) of HLA-DR expression on NPS and blood mDCs was compared in the RSV and control groups. HLA-DR expression on NPS mDCs was significantly lower in the RSV group compared with healthy controls (MFI 128 vs 408; p=0.003). Similarly, blood mDCs from RSV patients had diminished surface HLA-DR expression compared with blood mDCs from controls (MFI 70 vs 181; p=0.005). Expression of HLA-DR on NPS mDCs was significantly increased after resolution of RSV in 11 patients who returned for follow-up one month after infection (MFI 111 versus 175; p=0.02). Likewise, blood mDCs from RSV patients displayed increased surface HLA-DR one month later (MFI 65 vs 211; p=0.02). This depression of Class II MHC on mDCs, also observed on the plasmacytoid dendritic cells and monocytes in RSV patients, was reproduced *in vitro* by exposing PBMCs to live RSV. These data suggest that RSV employs downregulation of Class II MHC as a strategy of immune evasion.

 # 11 Section: Immunology – Innate and Adaptive **OR** Pathogenesis

Title: Immune evasion of respiratory syncytial virus by a viral neutralization decoy

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Abstract: Human respiratory syncytial virus (RSV) is unusual among common human pathogens in that it can infect very early in life despite the presence of virus-neutralizing maternal antibodies, with the peak of serious disease occurring at the remarkably young age of 2 months of life. RSV also has a marked ability to re-infect throughout life. The major RSV attachment surface glycoprotein G is expressed both in membrane-bound and secreted forms. Using recombinantly-generated viruses, we found that a mutation that eliminated expression of the secreted form of G rendered RSV substantially more sensitive to neutralization by RSV-neutralizing polyvalent and G-specific antibodies. This effect was observed both in vitro and in mice that had received passively transferred G-specific or convalescent RSV-neutralizing antibodies. These data suggest that the secreted form of the G protein helps the virus evade neutralization, presumably by functioning as a decoy molecule that binds virus-specific antibodies. This mechanism of immune evasion, which is not present in other common respiratory viruses, may help explain the unusual epidemiology of RSV. A number of other enveloped viruses also are known to produce secreted versions of their major virion surface glycoproteins, including Ebola virus, human immunodeficiency virus, and rabies virus, suggesting that they also employ the strategy of a neutralization decoy.

 # 12 PATHOGENESIS I SECTION

RESPIRATORY SYNCYTIAL VIRUS INFECTS AND MATURES PRIMARY MYELOID AND PLASMACYTOID DENDRITIC CELLS

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The inability of respiratory syncytial virus (RSV) to induce long-term protective immunity may be due to suppression of type I IFN and modification of T cell responses, suggesting events occurring at the level of antigen-presenting cells are critical to RSV pathogenesis. We therefore examined the ability of RSV to infect and activate myeloid dendritic cells (mDCs) and plasmacytoid DCs (pDCs). We demonstrate that RSV infects mDCs at greater rates (5%-20%) than pDCs (<5%). While <20% of each cell type was infected by RSV, most cells were matured as evidenced by increased expression of CD80/86 and cytokine/chemokine production. Divalent cations are required for DC infection and maturation. In contrast, UV-inactivated virus induced DC maturation in the absence of productive RSV infection. RSV has been shown to affect the macrophage response to LPS. To evaluate the impact RSV on the function of primary DCs maturation markers and cytokine production was examined after RSV infection and compared to increases seen following stimulation with LPS or R848, TLR4 and TLR7/8 agonists, respectively. Pre-exposure to RSV did not consistently interfere with the ability of TLR agonists to induce DC maturation and cytokine production. Because RSV G is secreted from cells within 6 hours of infection and may provide a mechanism to impact the local environment, we examined the effect of RSV G pretreatment on DC maturation. While DC maturation was induced, expression of CD40 and CCR7 was inhibited by treatment of mDC and pDC before RSV infection. Prior exposure of mDCs or pDCs to RSV G increased the production of selected chemokines (MIP-1alpha/beta or RANTES), but inhibited IFN-alpha, suggesting that more than one signaling pathway is affected. Therefore, even though direct infection of primary DCs does not consistently alter the responses to TLR ligands, secreted G (e.g. from neighboring infected epithelial cells) may indirectly affect primary DC function and have a significant impact on subsequent immune response and pathogenesis.

 # 13 Section: Immunology – Innate and Adaptive

Title: Suppressor of Cytokine Signaling (SOCS) Proteins Regulate the Type I Interferon Response to Respiratory Syncytial Virus (RSV) Infection through a Toll-like Receptor (TLR) Pathway

Authors: Christine M. Oshansky[1], Thomas M. Krunkosky[2], Les P. Jones[1], and Ralph A. Tripp[1]

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Abstract:
Respiratory syncytial virus (RSV) proteins contribute to immune evasion by modifying anti-viral cytokine and chemokine responses. The SOCS family of proteins includes eight members whose expression negatively regulates cytokines and chemokines. Here, we examine the role of SOCS-1 and -3 regulation of the type I interferon (IFN) response to RSV infection in primary fully differentiated normal human bronchial epithelial (NHBE) cells. The results show that SOCS-1 and -3 are increased during the adsorption phase of virus infection, i.e. within 15 minutes, and that increased SOCS expression is associated with decreased type I IFN expression. We show that the rapid activation of SOCS-1 and -3 is linked with RSV F protein signaling through pattern recognition receptor signaling pathways indicated by interferon regulatory factor (IRF)-3 hyperphosphorylation early following infection. This hyperphosphorylation occurs even in the absence of replicating virus. Moreover, utilizing confocal microscopy, we show that toll-like receptor (TLR)-4 is expressed on the surface of NHBE cells, and hypothesize that because the RSV F protein has been shown to interact with TLR-4, RSV modulates the type I IFN response through a TLR pathway before the known IFN antagonism by NS protein expression, suggesting that this feature may be an important mechanism to aid virus replication.

 # 14 Section: Pathogenesis.

Title: A critical role for poor Toll-like receptor stimulation and antibody affinity maturation in the pathogenesis of enhanced respiratory syncytial virus disease.

Authors: M. Florencia Delgado[1]; Silvina A. Coviello[1]; Jose A. Melero[2]; Alfonsina Trento[2]; Steven R. Kleeberger[3]; Fernando P. Polack[1, 4].

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Abstract: In the 1960s, a formalin inactivated vaccine against RSV (FIRSV) was administered to infants and children in the United States. FIRSV produced abundant non-protective antibodies with poor neutralizing capacity. Upon subsequent exposure to RSV, immunized children developed a severe lung disease characterized by bronchoconstriction and pneumonia. This enhanced disease (ERD) was associated with Th2 polarization of the immune response and immune complex deposition in affected tissue. Even though the phenotype of ERD has been extensively characterized, the mechanism by which antibodies generated by FIRSV failed to confer protection is not well understood. In fact, RSV vaccines inactivated by other methods also failed to elicit a vigorous protective antibody response. Had FIRSV elicited protective antibodies, exposure to RSV would not have caused this serious illness.
Using a murine model of ERD we demonstrate that affinity maturation of the antibodies against RSV is critical for protection and that failed affinity maturation after immunization with inactivated RSV vaccine resulted from poor stimulation of toll like receptors (TLRs). Vaccines inactivated by different methods failed to activate TLRs and therefore produced immature, pathogenic antibodies. To confirm these observations we interrupted affinity maturation after inoculation of mice with a protective live RSV vaccine and generated non-protective, pathogenic antibodies. In addition, TLR stimulation simultaneously with inactivated RSV inoculation elicited protective antibody against the virus. Poor activation of TLR was critical in the pathogenesis of ERD. Development of protective, high affinity, neutralizing antibodies against RSV is an important consideration for the development of candidate vaccines against this paramyxovirus.

15 Section: Immunology – Innate and Adaptive

Title: Respiratory Syncytial Virus and Human Metapneumovirus Inhibit Macrophage Interferon-γ Signaling through Increased Phosphorylation of STAT1β

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Abstract: Interferon γ (IFNγ) is a potent regulator of macrophage antimicrobial functions. We investigated the effect infection with the common paramyxoviruses, respiratory syncytial virus (RSV) or human metapneumovirus (hMPV), had on the macrophage response to IFNγ mediated activation. RSV and hMPV, but not UV-inactivated virus, inhibited the IFNγ stimulated luciferase expression from reporter plasmid driven by the γ-activated sequence response element (pGAS) in RAW264.7 (RAW) macrophages. Impaired pGAS-driven luciferase expression was not due to impaired STAT1 activation as phosphorylation of STAT1 in response to IFNγ was not reduced in RSV or hMPV infected RAW macrophages when compared to mock-infected RAW macrophages. Nuclear translocation of pSTAT1, as determined by confocal immunofluorescence microscopy and Western blot analysis of nuclear fractions, was similar in RSV-infected and mock-infected RAW macrophages. Interestingly, both RSV and hMPV infection increased the phosphorylation of STAT1β, a dominant-negative STAT1 splice-variant, in a time and dose dependent manner in response to IFNγ. Increased phosphorylation of STAT1β resulted in decreased nuclear STAT1 interaction with CBP/p300 and reduced transcription of the class II transactivator following IFNγ stimulation. These results suggest that RSV and hMPV modulate macrophage antigen processing and presentation through transcriptional repression of IFNγ stimulated genes.

 # 16 Section: Pathogenesis II and Pulmonary Aspects of RSV

Title: RSV Disrupts “Mechanical” Innate Defense of the Conducting Airways: Implications for RSV Infection in Chronic Lung Disease

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Abstract: RSV is associated with more severe and prolonged disease in patients with Cystic Fibrosis (CF) or other underlying chronic lung diseases (e.g., COPD, asthma). Previously we have shown that RSV targets ciliated cells in an *in vitro* model of human ciliated airway epithelium (HAE) and that ciliated cells derived from non-CF or CF airways are equally susceptible to RSV infection. Ciliated cells are present throughout the conducting airways and effective cilial beat functions to facilitate airway clearance of inhaled pathogens/particulates away from the fragile distal/alveolar regions of the lung. As such, ciliated cells are an important component of “mechanical” innate host defense against respiratory pathogens including RSV. We determined the pathophysiological consequences of RSV infection on ciliated cell function and the potential implications for RSV pathogenesis. Although RSV infection of ciliated cells was non-cytopathic for up to 2 days pi, after 3 days, cilial beat ceased and by 5 days pi, infected ciliated cells were extruded from the epithelium by a process resembling ‘anoikis’ in which infected cells only became apoptotic after shedding. Accumulations of dead and dying cells on the apical surfaces of HAE were combined with excessive mucus secretions also stimulated by RSV infection. These consequences of RSV infection were not quantitatively or chronologically different when non-CF or CF ciliated cells were infected. However, within the first 24 hrs of virus infection, non-CF HAE increased fluid secretion onto the apical surface of HAE that enhanced cilial beat effectiveness. This response was due to a direct effect of RSV on the ion transport capacity of ciliated cells and likely represents a host cell response to flush virus away from the epithelium thus facilitating clearance. In contrast, CF HAE failed to induce fluid secretion after RSV due to the absence and/or dysregulation of the ion transport properties of CF ciliated cells. In conclusion, we have modeled the pathophysiological consequences of RSV infection of human ciliated cells *in vitro* and speculate that in otherwise healthy airways, RSV-induced ciliated cell shedding and mucus secretions are accompanied by increased fluid secretion into the airway lumen facilitating mucociliary clearance of progeny virions and associated debris in an attempt to avoid distal spread of virus. However, in CF airways, RSV infected ciliated cells fail to sufficiently hydrate shed material leading to reduced clearance, prolonged retention times of virus and increased risk of distal spread of virus. Such events may precipitate exacerbation of chronic lung disease with more severe consequences of RSV infection.

 # 17 Section: Pathogenesis II and Pulmonary Aspects of RSV

Title: Co-Circulating Antigenic Subgroup A Strains of RSV Induce Differential IL-13, Mucus, and Disease Severity

Authors: Michael H. Chi[1], Martin L. Moore[1], John V. Williams[2], Peter F. Wright[2], James E. Crowe[2], Jr., Kathryn M. Edwards[2], Robert D. Collins[3], Vasiliy V. Polosukhin[1], Kasia Goleniewska[1], and R. Stokes Peebles, Jr. [1]

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Abstract: We tested the hypothesis that RSV strain differences are important for the immunologic features of RSV infection. RSV A/Nashville/2001/2-20 strain was derived from a nasal wash collected in February 2001 from a child with RSV lower respiratory tract infection. RSV A/Nashville/2001/3-12 strain was derived from a nasal wash collected in March 2001 from a child with RSV upper respiratory tract infection. These antigenic subgroup A RSVs were passaged nine times in HEp-2 cells by limiting dilution, followed by two rounds of amplification in HEp-2 cells. Plaques were visualized with anti-RSV F Antibodies. Sequencing of glycoproteins revealed these are distinct strains. BALB/cJ mice were mock-infected or infected with 10^5 PFU of A2, Long, line 19, A/Nashville/2001/2-20, or A/Nashville/2001/3-12 strains. A/Nashville/2001/2-20 infection resulted in greater lung IL-13, gob-5, and mucus expression as well as greater disease severity than the other RSV strains. Histopathologic findings in the lungs of BALB/cJ mice infected with A/Nashville/2001/2-20 included inducible bronchus-associated lymphoid tissue (iBALT), mucus, epithelial damage, smooth muscle damage, and smooth muscle hyperplasia, resembling the histopathology of severe RSV infection in infants. These data demonstrate that differences among co-circulating RSV strains have an impact on RSV pathogenesis.

 # 18 Section: Immunology-Innate and Adaptive

Title: Role of Human Metapneumovirus Glycoprotein G and Small Hydrophobic SH Protein on Chemokine and Type I Interferon Induction in Airway Epithelial Cells

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Abstract: Human metapneumovirus (hMPV) is a leading cause of acute respiratory tract infection in infants, as well as in the elderly and immunocompromised patients. Recently, a recombinant virus lacking G protein (rhMPV-ΔG) has been shown to be attenuated in the lung and upper respiratory tract, whereas a virus lacking the SH protein (rhMPV-ΔSH) replicates similarly to its wild-type counterpart. However, the role of these two proteins in modulation of hMPV-induced cellular responses in vitro, as well as in vivo, was not investigated. Here, we compared airway epithelial cell responses to infection with either recombinant wild type hMPV (rhMPV-WT) or deleted mutant viruses. Our results show that rhMPV-ΔG-infected airway epithelial cells produced higher level of chemokines and type I interferon (IFN) than those infected with rhMPV-WT, through enhanced activation of transcription factors belonging to the nuclear factor-(NF)-κB, interferon regulatory factor (IRF) and activator protein-1 (AP-1) families. Infection of airway epithelial cells with rhMPV-ΔSH resulted in enhanced secretion primarily of interleukin (IL)-6 and IL-8, two NF-kB-dependent genes, compared to rhMPV-WT. Western blot analysis showed increased nuclear levels of phosphorylated and acetylated NF-kB, following infection with rhMPV-ΔSH, compared to wild type virus, indicating a role of SH protein in modulating NF-kB activation. In summary, both SH and G proteins affect hMPV-induced NF-κB-dependent signaling, while G protein also modulated IRF and AP-1 activation, suggesting a role of G protein in regulating an early signaling event triggered in response to hMPV infection of airway epithelial cells. In vivo studies are needed to determine whether enhanced lung inflammation occurs following infection with these possible vaccine candidates.

 # 19 Section: Pathogenesis II and Pulmonary Aspects of RSV

Title: Respiratory syncytial virus reinfection is not associated with post-bronchiolitis wheeze

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Abstract: *Background:* Respiratory syncytial virus (RSV) lower respiratory tract infection (LRTI) is followed by post-bronchiolitis wheeze in 40-50% of cases. Literature suggests that neonatal RSV infection in mice predisposes to airway morbidity during RSV reinfection. We aimed to investigate whether RSV reinfection is associated with post-bronchiolitis wheeze in infants.
Methods: 73 infants were followed for 6 months after hospitalization for RSV bronchiolitis. Parents reported respiratory tract symptoms and during the first 3 episodes of respiratory tract symptoms undiluted nasopharyngeal aspirates (NPA) were taken. Wheezing and non-wheezing episodes were distinguished. Aspirates were analyzed for viral respiratory pathogens by using RT-PCR and for cytokines by using ELISA and Luminex.
Results: During 86 episodes of respiratory tract infections NPA were taken in 50 children of which 17 (20%) were associated with wheeze. In 98% respectively 53% of episodes one or more than one respiratory virus was detected. There were no differences found in viral respiratory pathogens in episodes with wheeze and episodes without wheeze. RSV reinfections were found in 2 (12%) children during episodes with wheeze and 9 (14%) children during episodes without wheeze. 27 cytokines were measured in NPA and 19 cytokines could be identified in all children. IFN-γ concentrations were significantly lower during wheezing episodes than non-wheezing episodes.
Conclusion: The results of this study show that RSV reinfection or persistence is not associated with post-bronchiolitis wheeze. Inability to sample the lower airway of infants may explain the discrepancy between the current study and previous murine studies. Unexpectedly, we found multiple viral respiratory pathogens in more than half of the episodes following RSV LRTI. Finally, a novel finding of this study is that post-bronchiolitis wheeze appears to be associated with a locally decreased Th1 response.

This work was funded by: Dutch Asthma Foundation

 # 20 Section: Immunology-Innate and Adaptive

Title: Age-related Differences in Pulmonary Cytokine Response to Respiratory Syncytial Virus Infection: Modulation by Anti-inflammatory and Antiviral Treatment

Authors: Marina S. Boukhvalova, Kevin C. Yim, Katie H. Kuhn, John P. Hemming, Gregory A. Prince, David D. Porter, and Jorge C.G. Blanco

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Abstract: BACKGROUND: Respiratory syncytial virus (RSV) is the major cause of severe lower respiratory tract infection in infants and young children. Recently RSV has also been recognized as a serious health risk in the elderly, but the pathogenesis of RSV infection in the elderly remains unknown. METHODS: Dynamics of pulmonary cytokine response (including IFN-γ, IL-4, IL-10, IL-6, MCP-1, and GRO mRNA) during acute RSV infection were investigated in young (<2 months old) and aged (>9 months old) cotton rats *S.hispidus*. Therapeutic treatments that diminish viral replication (antiviral antibody) and pulmonary pathology (anti-inflammatory corticosteroid) in RSV-infected animals were used to evaluate contribution of virus replication and inflammation to the development of RSV disease with respect to age. RESULTS: The time of the peak expression of majority of cytokines was shifted with respect to age. Antiviral and anti-inflammatory treatments had similar effect on cytokine expression in aged and young animals. GRO mRNA transcripts were more abundant in the lungs of aged animals. CONCLUSIONS: The present work reports the age-related delay in the pulmonary cytokine response to RSV, imbalance in chemokine production with respect to age and underscores different components of RSV pathogenesis with respect to their molecular signature.

#21 Section: Immunology – Innate and Adaptive

Title: Messenger RNA profiling of the innate response of lung epithelial cells to a set of respiratory viruses.

Authors: Arno C. Andeweg[1], Leon de Waal[1], Helma Vos[1], Leontine van der Wel[1], Maarten Bijl[1], Wilfred van IJcken[2], Rik L. de Swart[1], Roel Bakker[1], Albert D.M.E. Osterhaus[1]

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Abstract: As part of a multi-center genomics research program on host-respiratory virus interactions we characterized the innate host response of A549 lung epithelial cells to infection with five viruses (RSV, hMPV, influenza virus, PIV, and measles virus). In a time course experiment (0-24 hours) cells were exposed to live or UV-inactivated virus preparations. Collected samples were subjected to genome-wide mRNA profiling analysis. Microarray analysis (Affymetrix) demonstrated a tightly regulated host response with a large variation in the number of differentially expressed genes (0-1800 genes, mostly up-regulated). RSV, hMPV and influenza virus infections induced the strongest responses, whereas influenza virus infection rapidly down-modulated host mRNA levels. An immune related core response was identified in which not a single opposite/counter regulated gene could be recognized. Differences in the observed expression profiles related to the phylogenetic distance of the studied viruses. Relatively few genes were differentially expressed upon exposure to UV-inactivated viruses, again influenza virus infection being exceptional: broad innate immune responses were found at the mRNA level in the absence of virus replication. Global testing for specific biological pathways or processes so far did not reveal well characterized pathways that were exclusively triggered by a particular virus or condition; however, many genes displayed condition-specific expression patterns. For the viruses studied, an immune process-related, NF-kB controlled, core response could be identified. Currently the transcriptional control of the tightly regulated responses is reconstructed by bioinformatics reverse engineering. When focusing on selected (sub)pathways of the innate immune response all viruses appeared to induce multiple steps of this response despite specific viral host response interference mechanisms that are operational. The collected data set together with the analysis tools and approaches that are currently being developed serve as a platform for in depth studies on the virus-host interaction currently performed in *in vitro* and *in vivo* model systems for respiratory virus infections.

 # 22 Section: Immunology – Innate and Adaptive

Title: Early responding CD8+ T cells are critical in the resolution of pulmonary eosinophilia in a murine model of respiratory syncytial virus vaccine-enhanced disease.

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Abstract: Respiratory syncytial virus (RSV) vaccine-enhanced disease in mice can be generated by vaccinating mice with a vaccinia virus vector containing the RSV G protein followed by intranasal RSV infection. This process is characterized by a potent memory CD4+ T cell response with type II features including an enhanced pulmonary eosinophil infiltrate without the simultaneous induction of a memory CD8+ T lymphocyte (mCD8+) response. In this study, we used two models to investigate how and when the generation of a mCD8+ T cell response may affect the development of pulmonary eosinophilia and the progression of vaccine-enhanced disease. First, we adoptively transferred various RSV-specific and non-specific purified CD8+ T cells into G-primed mice at different times during the course of a RSV challenge infection. Secondly, we used a depletion antibody at various times post-RSV infection to eliminate CD8+ T cells in mice that could generate both mCD4+ and mCD8+ T cell responses. By assessing the levels of pulmonary eosinophilia evident in both models, our results suggest that the induction of a RSV-specific mCD8+ T cell response early following RSV infection can attenuate pulmonary eosinophilia and thus prevent the development of vaccine-enhanced disease.

#23 Section: Vaccines and Clinical Aspects of RSV

Title: Detection and Spread of RSV in Young Children Attending Daycare

Authors: Janet A. Englund[1,2], Emily Martin[1,2], Jane Kuypers[2], Susan Chambers[1,3], and Mary Fairchok[1,2,3]

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Abstract: Increased rates of respiratory tract infections (RTIs) occur in daycare attendees, but rates of RSV-related disease in this setting has not been explored using modern molecular detection methods.
Methods: Children 1-30 months (M) of age were enrolled at 2 daycare centers. From Feb. 2006-Mar. 2007, nasopharyngeal swabs were obtained from each child for each illness episode using a nylon flocked swab (Copan Diagnostics, Corona, CA). RT-PCR was performed to detect 14 common pediatric respiratory viruses, including RSV. Symptom diaries were completed by caretakers for up to 10 days following the onset of each child's illness. The two-month failure rate in each daycare classroom following documentation of the initial RSV infection was then calculated using the Kaplan-Meier method.
Results: We prospectively followed 101 children (mean age 11 M; range 1.5-25 M) for 56.5 child-years. The mean annualized incidence of RTIs was 6.2 episodes /child; children had RTI symptoms an average of 20% of the time. We detected at least one virus in 61.5% RTIs. Altogether, 28 RSV infections were detected for an incidence of 49.5 per 100 child-years. RSV had the largest clinical impact of all respiratory viruses detected and was associated with more clinic visits ($p<0.001$), fever ($p= 0.002$), and parental absenteeism (1.8 days; $P<0.02$). At least one RSV infection was observed in children attending 7 of 13 separate rooms. Subsequent cases of RSV were documented among study participants in 4 of 7 rooms, with secondary cases occurring from 1 to 10 days later. The Kaplan-Meier failure estimates in each room 2 months after the first documented RSV case were 31%, 48%, 55%, and 57% (95% C.I.: 13%-63%; 28%-73%; 22%-92%; and 35%-80%, respectively).
Conclusions: RSV was not the most common virus identified in young children attending daycare, but had the greatest clinical impact in these young children and their families. Spread of RSV within individual classrooms was rapid.

This study was sponsored in part by MedImmune, Inc.

#24 Section: Vaccines and Clinical aspects of RSV

Title: Differential gender response to respiratory infections and to the protective effect of breast milk in premature infants.

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The protective role of breastfeeding against severe acute lung disease in infants is well-established, but its mechanism is unclear. Most hypotheses assume that breastfeeding confers similar passive protection to every infant. However, a few observations suggest that the benefits of breast milk against severe lung disease may differ by gender.

We conducted a prospective cohort study designed to determine the role of gender and breastfeeding on susceptibility to severe acute lung disease among intensively monitored high risk infants.

One-hundred and nineteen high risk infants were enrolled in the study. Breast milk protected females against severe acute lung disease (risk in breastfed = 6.5% vs. 50% in formula-fed; p=0.001), but not males (risk= 18.9% vs. 18.5%; p= 0.97). The interaction between breastfeeding and gender was clinically and statistically significant (p=0.011), even after adjustment for variables that can affect severity of acute lung disease (p=0.008). Disease was most severe in formula-fed girls (p=0.01 vs. formula-fed boys). Breastfeeding decreased the risk of severe acute lung disease in girls, but not in boys. These findings suggest that breast milk protection is not conferred by passive transfer of humoral immunity (which should be gender-indifferent), show that respiratory symptoms may be amenable to non-specific modulation, and identify non-breastfed premature infant females as an at-risk group for severe acute lung disease.

#25 Section: Vaccines and Clinical aspects of RSV

Title: RSV F, G and N proteins bind to neutrophils in Bronchoalveolar lavage fluid (BAL) and blood of infants with severe RSV Bronchiolitis.

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Abstract: A high proportion (≈85%) of the cellular infiltrate in the airways of infants with RSV Bronchiolitis are neutrophils. We investigated whether RSV binds to these neutrophils.
Methods: Following informed consent blood and BAL samples were obtained from 20 infants RSV positive infants with severe Bronchiolitis and 7 controls, aged less than 18 months, immediately after intubation. Infants with congenital cardiac or respiratory disease were excluded. Neutrophils, enriched from samples using sedimentation gradient techniques, were analysed by flow cytometry (FACs) and immunocytochemistry. Each FACs assay was triple labelled with CD16 PE, Ethidium Monoazide Bromide (EMA) and RSV F, G or N proteins.
Remaining neutrophils were dual labelled with c-ANCA-FITC and RSV F, G or N-PE and visualised with a fluorescent camera.
Results: The samples contained >95% neutrophils.
The percentage of neutrophils in the BAL of infants with severe Bronchiolitis positive for RSV F,G and N proteins were 83%(74,88), 74%(44,88) and 80%(42,88) (median percentage ,IQR)) respectively. This compared with <3% (1, 5), for all three proteins, in controls, P<0.05. In the blood, F,G,N RSV proteins were detected in 41%(18,74), 38%(12,73) and 37%(12,72) respectively of the neutrophils from cases compared to < 1%(0.5,1.5) in controls, P<0.05. Using fluorescent imaging RSV F, G, N protein was seen within the cytoplasm of neutrophils in the blood and the BAL of cases but not in controls.
Conclusions: RSV F, G and N protein can bind to neutrophils in the blood and the BAL of infants with severe RSV infection. This suggests, in infants with severe RSV Bronchiolitis, whole virus is binding to neutrophils in the lungs and that RSV causes a viraemia.

26 Section Clinical and Vaccines

Title: The Frequency and Severity of Wild-Type Respiratory Syncytial Virus (RSV) Infection Occurring After Receipt of Live, Attenuated, RSV Vaccines.

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Background: Early in the development of RSV vaccines severe disease occurred in some children after receipt of formalin-inactivated RSV vaccine. The illnesses occurred in children who had not previously experienced RSV infection but had wild-type RSV infection in the winter season subsequent to vaccination. Live attenuated, intranasally administered vaccines provide a broadly based immune response and, by mimicking the response to natural infection, are unlikely to result in potentiated disease. Continuing efforts to develop an appropriately attenuated and immunogenic live RSV vaccine have provided an opportunity to give assurances that live vaccines are a safe approach to RSV immunization and to provide data on optimal design of subsequent vaccine efficacy trials.
Methods: Between 1996 and 2003 7 different RSV vaccines were given to 113 6-24 month old children seronegative to RSV at immunization and 2 vaccines to 62 RSV-naïve infants 1-3 months of age. In total, 388 vaccinees, placebo recipients and age-matched controls were followed for respiratory illness during the subsequent winter. Nasal wash samples were cultured for RSV with each episode of respiratory disease. For purposes of analysis we focused on: 1) a comparison between all vaccinees and controls - an "intent to treat" analysis, and 2) for two vaccines given to both 6-24 month olds and 1-3 month olds - a comparison of those who had a response to vaccination (evidence of virus shedding or an immune response) versus controls - a "vaccine efficacy" analysis. Pre and post-season sera available on 122 of the infants and 143 of the children were examined for serologic evidence of RSV infection.
Results: In the "intent to treat" analysis the rate of RSV-associated upper respiratory tract illness was lower in vaccinated children than in controls (14% versus 20% in the 6-24 month old group and 16% versus 25% in infants). RSV-associated lower respiratory tract illness was infrequent and there was no evidence that vaccination predisposed to more severe illness. The vaccine "efficacy" analysis of the two leading vaccine candidates (cpts 248/404 and rA2cp 248/404/1030/ΔSH) revealed similar trends towards efficacy. RSV serologic rises over the ensuing winter were common in children (31%) and infants (21%) regardless of receipt of vaccine or placebo.
Conclusions: This surveillance conclusively demonstrated that infection with a live attenuated RSV vaccine did not lead to enhanced disease upon infection with wild type RSV. The information on the impact of RSV in the young infants and children during the period of surveillance will assist in designing future efficacy studies with RSV vaccines.

#27 Vaccine and Clinical Aspects of RSV

Adult Human Metapneumovirus (hMPV) Infections: Epidemiology and Clinical Impact. Edward E. Walsh, Maryanne Formica, Gloria Andolina, Mary Criddle, Patricia Henessey, Ann R. Falsey. Rochester General Hospital and University of Rochester School of Medicine and Dentistry, Rochester, NY.

Background: hMPV is a newly identified virus most closely related to RSV based on genomic and protein structure. The clinical importance of this virus among adults has been less well studied than in infants.

Methods: All respiratory illnesses in 825 community dwelling adults ≥ 18 years of age (range 18-100) were evaluated for respiratory viral infections during a 4 year period (1999-2003). Subjects were categorized as healthy old (n=622), high-risk (n=528), and young (n=297), and were followed for two consecutive winters (Nov-Apr). In addition, hospitalized patients (n=1481) with acute respiratory symptoms were enrolled and evaluated. Infection with hMPV (as well as RSV, influenza A, and coronaviruses) was confirmed by RT-PCR and serology. Clinical data was compared to RSV and influenza A infections in the same population.

Results: A total of 2,988 respiratory illnesses were identified, of which 94% were fully evaluated. Overall, 914 viral infections (33% of all illnesses) were documented. Excluding mixed infections, hMPV represented 14.7%, RSV 22.4%, and influenza A 21.8% of the viral illnesses. Overall, hMPV infection rates in the prospective outpatient cohorts ranged from 5.9% to 12.8%. Uniquely for hMPV, young adults had asymptomatic infection more commonly than symptomatic infection. Among hospitalized subjects, hMPV was the 3rd most commonly identified virus (7.5% of illnesses) behind influenza (10%) and RSV (9.5%). Clinical hMPV illness was similar to RSV and subjects were more likely to have wheezing than with influenza A. ICU care, ventilation rates and mortality were similar for these three pathogens.

Conclusion: hMPV appears to be the third most important viral pathogen among adults, especially elderly and high-risk persons. Although asymptomatic illness is common, a significant impact can be seen among hospitalized persons. Control measures, such a vaccine development, may be warranted. The protective effect of antibody on severity and incidence of infection is currently under investigation.

#28 Section: Vaccines and clinical aspects of RSV

Title: Vaccination approaches to combat human metapneumovirus lower respiratory tract infections

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Abstract: Human metapneumovirus (hMPV), a paramyxovirus discovered in 2001, causes acute respiratory tract illness in young children, individuals with underlying disease and the elderly. We recently showed that infection with hMPV induces only transient protective immunity, which could impede vaccine development. Here we describe the evaluation of 2 vaccine candidates, a live-attenuated virus vaccine and a soluble F subunit vaccine in Syrian golden hamsters (*Mesocricetus auratus*).

Cold-adaptation (ca) of hMPV in Vero-cells resulted in the accumulation of temperature sensitive (ts) mutations in the viral genome. Using reverse genetics, a recombinant virus containing these mutations was constructed which displayed a low shut-off temperature in vitro. Replication of this virus was reduced in the upper respiratory tract (URT) of hamsters, and no virus replication was detected in the lower respiratory tract (LRT). Nevertheless, high titres of hMPV-specific antibodies were induced. Upon immunization with the ca/ts-virus, the LRT of hamsters were completely protected against infection and URT viral titres were 10.000 fold reduced.

Soluble F subunit vaccines were produced in mammalian cell cultures. Immunization of hamsters with adjuvanted soluble F proteins induced high virus neutralizing antibody titers, with higher titers against the homologous than the heterologous virus. Animals were protected from subsequent LRT infection with homologous and heterologous viruses. URT viral titres were also significantly reduced in immunized animals.

We conclude that immunization against hMPV with soluble F vaccines or live-attenuated vaccines induces protection against LRT infections in hamsters.

#29 Section: Immunology – Innate and Adaptive

Title: Small Interfering RNA (siRNA) Prophylaxis Reduces RSV Replication in Mice and Enhances the Immune Response: Prospects for Vaccinology

Authors: Wenliang Zhang and Ralph A. Tripp

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Abstract:

Respiratory syncytial virus (RSV) is a major cause of morbidity in infants and young children worldwide. Currently there is no effective vaccine or anti-viral drug to control infection. RNA interference (RNAi) is a powerful tool amenable to development of anti-viral drugs. Our research has shown that synthetic small interfering RNAs (siRNA), which mediate RNAi, robustly silence RSV replication in vitro and in a BALB/c model of RSV infection. Here, we examine the influence of siRNA prophylaxis on the primary and memory immune response to RSV infection in mice. We show that mice treated with siRNA targeting the RSV P gene exhibit reduced RSV replication, pulmonary inflammatory cell infiltrates, and lung pathogenesis, and exuberant pulmonary memory T cell responses compared to mice treated with a siRNA control. The pulmonary T cell memory response revealed high frequencies of activated ($CD44^{hi}/CD62L^{lo}$) $CD4^{+}$ and $CD8^{+}$ T cells, M2 peptide-tetramer^{+} $CD8^{+}$ T cells, and high frequencies of T cells expressing IFN-γ and IL-5. The results support the hypothesis that siRNA drugs are effective anti-viral drugs to reduce the viral load to a level that allows for the induction of potent memory responses suggesting that siRNA treatment may act as well to vaccinate against RSV infection.

 # 30 Section: Therapeutics

Title: Antiviral Activity of Leflunomide against RSV *in vivo*

Authors: Melinda Dunn[2], Debbie Knight[1], Joan Durbin[1,3], W. James Waldman[1]

Affiliations: The Ohio State University [1]Department of Pathology and [2]Integrated Biomedical Sciences, [3]Children's Research Institute, Columbus, Ohio

Abstract: Currently approved therapeutic options for treatment of RSV disease are limited to ribavirin, which has not been shown to change the outcome of infection and must be administered by aerosol for 3 to 7 days, and passive immunoprophylaxis. The immunosuppressive agent leflunomide, an inhibitor of protein kinase activity and pyrimidine synthesis currently in Phase I clinical trials in transplant recipients, has been shown to exert potent antiviral activity against several herpesviruses. We have previously demonstrated that A77 1726, the active metabolite of leflunomide, exerts antiviral activity against RSV *in vitro* in a dose dependent manner over a pharmacologically-relevant range of concentrations. We have now tested the hypothesis that leflunomide reduces viral load *in vivo*. Cotton rats were inoculated with RSV (strain A2 or each of 2 low passage clinical isolates) and treated daily with leflunomide by gavage (20-30 mg/kg). Animals were euthanized on day 5 post-inoculation (p.i.) and viral load was quantitated in lung homogenates by plaque assay. Data generated by these experiments showed a >3 log reduction in viral titer in leflunomide-treated animals compared with vehicle-treated controls. Importantly, viral load in lungs of animals in which leflunomide treatment was delayed until day 3 p.i. were reduced to the same degree as those in animals treated beginning on the day of inoculation, implying that even a short course of treatment (days 3 and 4 p.i.) can effectively control the virus. These findings suggest promise for leflunomide, which can be administered orally, as a convenient addition to the growing arsenal of antiviral therapeutics. While specific antiviral mechanisms remain to be elucidated, leflunomide shows unique bifunctional potential to both reduce viral load and attenuate inflammation associated with RSV disease.

#31 Section: Therapeutics

Title: Antiviral and anti-inflammatory activities of TMC353121, a potent antiviral fusion inhibitor, in a mouse model of RSV infection

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Abstract: TMC353121 is a novel small-molecule fusion inhibitor with potent *in vitro* activity against RSV. To test its effects *in vivo*, we compared pre-infection administration of TMC353121 and palivizumab in the BALB/c mouse model of RSV infection.

To determine antiviral potency, viral load was analyzed in bronchoalveolar lavage fluid (BALF) and lung tissue by quantitative RT-PCR (qRT-PCR) and plaque assay after intranasal RSV infection using A2 strain human RSV ($2x10^6$ pfu). Histopathologic examination of the lungs and total and differential BALF cell counts were used to assess the inflammatory reaction to infection.

A single injection of 0.25 mg/kg TMC353121 administered 1 hour prior to RSV infection caused a ~75 % reduction in viral load in lung and BALF viral titer; doses as high as 10 mg/kg were well tolerated and caused only slightly greater antiviral effects (80% reduction in viral load). The antiviral effect was similar or better than that of palivizumab, administered by i.p. at 50 mg/kg, on the day before viral challenge. In repeated daily dose studies, delaying administration of TMC353121 by up to 2 days after infection reduced viral load and lung inflammation on day 4.

RSV infected mice that were pretreated with TMC353121 at a dose of 2.5 mg/Kg had similar inflammatory scores to uninfected mice, indicating that TMC353121 limits cell infiltration into the lung following RSV infection. Interestingly, although 0.25 mg/kg inhibited viral replication equally, it did not completely abolish the inflammatory response to challenge. The possible anti-inflammatory effect of TMC353121 might be directly or indirectly related to the antiviral properties of the compound.

The observed antiviral and anti-inflammatory properties of TMC353121 in the BALB/c model of RSV infection suggests that the compound is a promising antiviral fusion inhibitor, suitable for further clinical development.

 # 32 Section: Therapeutics

Sym003: Fully human polyclonal antibodies for the prevention of RSV disease

Johan Lantto, Henriette S. Nielsen, Klaus Koefoed, Lucilla Steinaa, Peter S. Andersen, Per-Johan Meijer, Torben P. Frandsen, Finn C. Wiberg and Lars S. Nielsen, Symphogen A/S, Lyngby, Denmark

Symphogen has exploited the natural human antibody response for the development of Sym003; recombinant polyclonal, fully human antibodies for the prevention of RSV disease. Natural cognate pairs of antibody genes were isolated directly from immune-competent human donors using our proprietary Symplex™ Technology platform and the resulting antibody repertoires screened for anti-RSV activity. Maintaining the original antibody heavy and light chain paring ensures that the configuration and specificity of the resulting antibodies are preserved in an unaltered form.

Sym003 represents an entirely new class of antibody therapeutics against viral infections as it contains potently neutralizing, high-affinity antibodies against multiple distinct and biologically relevant epitopes on both the F and G proteins. Sym003 displays potent neutralization against both RSV subtypes *in vitro*. Likewise, superior *in vivo* efficacy over existing therapeutic mAbs has been established in validated animal models. Due to the dual targeting of the F and G proteins, Sym003 also has the potential to block the immunomodulatory effects of both surface proteins and thereby reduce RSV-associated complications more efficaciously than a mAb. Furthermore, the targeting of multiple epitopes on both antigens is likely to provide efficacious protection against diverse, naturally circulating viruses as well as minimize the potential long-term risk of drug escape, thereby reducing the risk of clinical failure. The natural origin of the Sym003 antibodies and the highly specific targeting of RSV antigens minimize any safety and immunogenicity concerns.

We present here an entirely novel approach to the development of antibody-based therapeutics, which holds promise for a more efficacious prevention of RSV disease.

33 Section: Therapeutics

Title: Immune-modulatory role of the Fc region of Motavizumab

Authors: Subramaniam Krishnan, Peter Kiener and JoAnn Suzich

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Abstract: Antibodies bound to infected cells interact with innate immune effector cells expressing Fc receptors thereby triggering phagocytosis, mediating antibody-dependent cellular cytotoxicity (ADCC) and release of inflammatory mediators including chemokines which orchestrate the nature of the adaptive immune response. We studied whether the humanized $IgG_1\kappa$ monoclonal antibody (MAb) motavizumab, that is directed against the antigenic A site on the respiratory syncytial virus (RSV) F protein, could mediate any of these processes when bound to RSV-infected epithelial cells. Motavizumab was derived by affinity maturation from Synagis®, a licensed MAb that is indicated for the prevention of serious lower respiratory tract disease caused by RSV in high-risk infants. In the present study, HEp-2 cells were infected with RSVA at a multiplicity of infection of 1.0. Motavizumab was added to the cultures along with IFN-γ activated human monocytic THP-1 cells at 12-15 hours post-infection. Cytokine and chemokine release from and phagocytic potential of THP-1 cells were assayed. Significant release of MIP-1β, MCP-1, IP-10 and Eotaxin-3 was observed in cultures with motavizumab as early as 6 hours after co-culture, though only MIP-1β was significant 24 hours after co-culture. Phagocytosis of infected epithelial cells by activated THP-1 cells was also observed in the presence of motavizumab. These effects were not observed with an irrelevant humanized $IgG_1\kappa$ control MAb, or with motavizumab Fab or Fab'2. These studies suggest that motavizumab could mediate clearance of virus-infected epithelial cells and the selective release of chemo-attractants involved in recruiting effector and memory $CD4^+$ and $CD8^+$ T cells to the site of infection through Fc binding to monocytes/macrophages.

#34 Section: Vaccines and clinical aspects

Title: Resistance of fresh isolates of human respiratory syncytial virus to neutralization by anti-F murine monoclonal antibodies, palivizumab and human serum.

Authors: Edna L. Michael Gias, Sarah Welsh, Soren U Nielsen and Geoffrey L. Toms

Affiliation: School of Clinical Medical Sciences, The Medical School, Newcastle University, Newcastle upon Tyne, UK.

Abstract: Fresh isolates of hRSV exhibit marked resistance to neutralization by anti-fusion protein monoclonal antibodies. Resistance is transient on propagation of the viruses in cell culture and this has hitherto hindered analysis of the mechanisms involved. In this study, clonal analysis of early passage, resistant virus stocks yielded predominantly resistant clones, although after three passages in cell culture a small proportion of clones were fully susceptible to neutralization. Late passage, susceptible stocks yielded clones which were predominantly susceptible although a minority retained resistance. Replication rates of susceptible clones were markedly higher than those of resistant clones indicating that this phenomenon is the result of a quasi-species shift in the virus population with the emergence and selection of rapidly replicating, neutralization susceptible variants in cell culture. The clones derived were phenotypically stable allowing further analysis of the mechanisms involved. Resistant clones were co-resistant and susceptible clones co-susceptible to neutralization and fusion inhibition by murine anti-F monoclonal antibodies and to neutralization by palivizumab and human sera. Sequence analysis revealed no consistent change in the F gene or G-F intra-genic sequences between resistant and susceptible clones. The phenotype was preserved for density gradient purified virions from each clone and F gene content per genome was similar for both resistant and susceptible phenotypes. However the genome:infectivity ratio for resistant clones was approximately two-fold higher. Addition of UV inactivated resistant virus but not susceptible virus to a susceptible virus stock rendered the latter resistant to neutralization. These data suggest that a virus specific product, lost on adaptation to cell culture, blocks the action of anti-F antibodies in fresh isolates of virus. This may have significance for the development of both passive and active prophylactic regimes.

#35 Section: Vaccines and Clinical aspects of RSV

Title: Gene expression profiling in RSV challenged mice after different priming regimes.

Authors: Leon de Waal[1], Leontine van der Wel[1], Maarten Bijl[1], Roel Bakker[1], Geert van Amerongen[1], Wilfred van IJcken[2], Rik L. de Swart[1], Albert D.M.E. Osterhaus[1] and Arno C. Andeweg[1]

Affiliations: [1]Department of Virology and [2]Erasmus Center for Biomics, Erasmus MC, Rotterdam, The Netherlands.

Abstract: The RSV BALB/c mouse model has been widely used to study vaccine-mediated immunopathology. We have primed mice with either live RSV or different recombinant vaccinia constructs (rVV) expressing the RSV F (rVV-F), G (rVV-G) or M2 (rVV-M2) protein. As controls, mice were either primed with wild-type vaccinia virus (VV-wt) or left unprimed. Three weeks after priming all mice were challenged and samples (serum, lungs and tracheo-bronchial lymph nodes) obtained from these mice were used both for traditional virological/immunological assays and mRNA expression profiling using Affymetrix mouse 2.0 chips.
Traditional read-outs showed that all specifically primed animals (RSV, rVV-F, rVV-G or rVV-M2) were protected from challenge and that only rVV-G primed animals produced RSV-specific IgG1. Phenotyping of broncho-alveolar lavage samples showed that only rVV-G primed animals had eosinophils infiltrating the lungs after RSV challenge. Messenger RNA expression analysis revealed low levels of gene expression variation within the groups and apparently tightly regulated host responses. Furthermore, we observed phenotype-specific expression profiles, the highest number of differentially expressed genes being observed in the animals primed with rVV-G. The number of differentially expressed genes was higher in the lungs than in the lymph nodes. Using predefined sets of marker genes for specific processes or cells (e.g. Th1 and Th2), the traditional read-outs could be confirmed with the micro-array analysis. We identified individual genes and gene expression profiles that are associated with or control phenotypically distinct host responses. The collected information allows the in depth study of responses to (candidate) vaccines at high resolution in relation to the potential predisposition for enhanced disease.

 # 36 Section: Vaccines & Clinical Aspects RSV Disease

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Affiliations: [1]University of Rochester School of Medicine, Rochester, NY; [2]Public Health Laboratory Service, London, England; [3]Brown University School of Medicine, Providence, RI; Eastern Virginia Medical School, Norfolk, VA; and [4]Sanofi Pasteur, Toronto, Canada.

Title: Safety and Immunogenicity of a Non-Adjuvanted RSV Vaccine Compared to RSV Vaccine Adjuvanted with Alum Given Concomitantly with Influenza A Vaccine in High-Risk Elderly

Abstract: RSV has been recognized as a significant pathogen in adults in recent years. The elderly and adults with underlying cardiopulmonary disease appear to be at highest risk for serious disease. The purpose of this two-year Phase II clinical trial was to compare humoral responses to licensed trivalent influenza vaccine given concomitantly with two RSV vaccine formulations, with and without alum. Secondary objects were to evaluate the safety and immunogenicity of the two RSV vaccines. Subjects were evaluated for acute respiratory infections (ARI) during each winter, and diagnoses of RSV using RT-PCR and/or ≥4-fold rise in N antibody by EIA and influenza by RT-PCR and/or HAI serology. In year one, 1180 participants were enrolled, of which 1169 were evaluable for ITT analysis. Safety profiles of both vaccines were clinically acceptable. There were no observable effects of either RSV vaccine on the level of anti-HI GMTs one month post-vaccination. Neutralization antibody titers (NA) for RSV and RSV alum vaccinees were greater than the 67th percentile of pre-vaccination distribution of NA titers in 77% and 78% of participants, respectively. The non-adjuvanted vaccine was judged to be superior based on $\log_2$ GMT and % of subjects with ≥ 4-fold rises (12.53 vs. 12.12 and 72% vs. 60%). At the time of the second vaccination, NA titers had returned to nearly baseline but again achieved a boost with unadjuvanted vaccine with 69% of participants achieving the 67th pre-vaccination percentile. The incidence of ARI associated with RSV and influenza A was low in both years. In year one, of 492 illnesses, 36 (7.3) were associated with RSV and 38 (7.8) with influenza A. Of 189 illnesses in year two, 12(6.4%) were RSV and 20 (10.7%) were influenza A. In year 1 the incidence of RSV infection in the RSV alum, RSV alone and placebo groups was 2.8, 3.1, 3.4 per 100 subjects respectively, and 3.1 and 1.1 per 100 subjects in RSV alone and placebo groups in year two. Although the safety and immunogenicity data of current RSV candidate are encouraging, the low rates of ARI associated with RSV make design of a large-scale efficacy trial challenging.

Poster Abstracts

#37 Section: Vaccines and Clinical aspects of RSV

Title: Development of a quantitative real-time diagnostic multiplex PCR assay for the detection of RSV A, RSV B and hMPV from clinical samples.

Authors: Adriana Alvarez-Aguero, Richard Allan and Alison Bermingham

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Abstract: Respiratory syncytial virus (RSV) is the leading viral cause of respiratory illness in infants and children worldwide and is associated with significant morbidity and mortality in the elderly. However, there is increasing evidence that RSV can cause infection in all age groups. Diagnosis of RSV cannot be reliably made based on clinical presentation alone as many pathogens causing respiratory infections are clinically indistinguishable. Laboratory diagnosis of RSV infection has traditionally relied on virus culture and immunofluorescent assays but rapid molecular methods for diagnosis have become more prevalent in recent years. Here we describe the development of a quantitative real-time multiplexed PCR assay for use in a diagnostic setting. We initially developed a real-time quadraplexed PCR assay to simultaneously detect RSV A, RSV B and hMPV in the presence of an internal extraction control. The multiplex assay was used to screen around 1500 combined nose and throat samples from a UK community surveillance scheme where around 3% of samples were RSV positive. The assay was then made quantitative by cloning each of the PCR diagnostic targets into a vector under the control of the T7 polymerase. T7 transcripts were generated, purified and used to make standard curves for their respective viral targets. Although the relationship between quantitative RSV viral load and disease severity remains unclear, initial analyses suggested highest viral loads were found in children and in the elderly. The use of a quantitative RSV diagnostic real-time PCR can therefore elucidate viral loads in specific age groups and potentially identify populations which would most benefit from intervention strategies for infection control or prevention.

38 Section: Vaccines and Clinical aspects of RSV

Title: Vaccination with recombinant Modified Vaccinia virus Ankara expressing bovine respiratory syncytial virus (bRSV) proteins protects calves against RSV challenge.

Authors: Adriaan F.G. Antonis[a], Robbert G. van der Most[b], Yasemin Suezer [c,d], Norbert Stockhofe-Zurwieden[a], Franz Daus[a], Gerd Sutter[c,d] , Remco S. Schrijver[a]

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Abstract: Respiratory syncytial virus (RSV) infections are a major cause of respiratory tract diseases during infancy in both calves and humans. The epidemiology and pathogenesis of bovine (b) RSV infection in calves closely resembles the human (h) RSV infection in young infants, and children. Vaccine development against hRSV has been hampered by a dramatic vaccine failure in the 1960s: vaccination with formalin-inactivated (FI), alum-adjuvanted virus predisposed children to an enhanced RSV disease after subsequent infection. This vaccine-induced immunopathology was reproduced in our natural-host bRSV model. BRSV models can therefore be used to obtain a deeper understanding of RSV disease in humans and vaccine-induced enhanced disease. We have evaluated the efficacy and safety of Modified Vaccinia virus Ankara (rMVA) based vaccine candidates, expressing the bovine RSV (bRSV) F protein, either or not in combination with the G protein, in colostrum deprived SPF calves born by caesarean section. Vaccination induced bRSV specific IgG and CD8 T cell responses. Importantly, no IgE responses were detected. After bRSV challenge, rMVA-vaccinated calves experienced less severe symptoms of lower respiratory tract disease compared to the mock-immunized control group. Immunized animals showed reduced pulmonary virus loads, and no eosinophilic infiltration or enhanced respiratory distress. In conclusion, candidate rMVA/bRSV vaccines induced protective and safe immune responses in calves.

#39 Section: Clinical aspects –MPV

Title: New Sub-Lineage Of Human Metapneumovirus In India Causing Acute Respiratory Infections In Children

Authors: Sagarika Banerjee[1], Shobha Broor[1] , Sushil . K. Kabra[2] , Preeti Bharaj [1], Wayne M Sullender[3]

Affiliations: [1] Deparrtment of Microbiology, All India Institute of Medical Sciences, [2]Department of Pediatrics, All India Institute of Medical Sciences, [3]Division of Pediatric Infectious Diseases, Department of Pediatrics, University of Alabama, Birmingham, AL

Abstract

Human metapneumovirus (hMPV) has been recognized as an important etiological agent of acute respiratory infections (ARI) in children as well as adults. There is paucity of information on genetic variability among circulating strains from developing countries. A preliminary report from Pune and from our laboratory suggested the potential importance of hMPV as a cause of ARI among children in India. Broad classification of hMPV based on fusion protein (F), nucleocapsid (N) has revealed 2 lineages viz. A and B that are divided into subgroups A1, A2, B1 and B2. Recently, a new sub-lineage that is bipartition of subgroup A2, designated as A2a and A2b has been reported from Germany (Huck *et al, 2006)*. Our aim was to characterize the circulating hMPV viruses in India by studying the nucleotide sequence variability in F and N genes. Nasopharyngeal samples were collected from 638 children <5 years of age presenting to AIIMS from April 2004 to April 2007 with ARI. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was used to detect hMPV using self designed primers for N and F genes. Partial N and F gene sequences of the hMPV positives were determined and phylogenetic analysis was performed.

Thirty four of 638 samples (5.3%) were positive for hMPV by F and N gene PCR. Sequence analysis of F gene sequences (400 nucleotides) from 26 positive samples showed that 60% of the hMPV strains clustered in the new A2b sub-lineage, 1 in B2 lineage and 9 clustered with the B1 lineage. Sequence analysis of N gene sequences (365 nucleotides) from 12 positive samples further confirmed the existence of the new A2b strains. Characterization of majority of Indian isolates into A2b sublineage was further supported by high bootstrap values of 93 and 99% for N gene and 99 and above 50% for F gene respectively. Analysis of partial F gene sequences showed 97.2-99.7 percent nucleotide identity among the Indian hMPV A2b isolates. The Indian A2b sequences showed 95.8-99.6% nucleotide identity with the German A2b sequences and 98.1-99.6% with the Japanese A2b sequences. This is the first report of the existence of the newly described A2b sublineage in India. In our 3 year study it was observed that during 2004-2005 winter-spring season A2b viruses circulated, in the next winter-spring season A2b and B1 strains co-circulated with A2b strains predominating and during 2006-2007 winter-spring season B1 strains predominated and 1 strain was found to be of B2 sub-lineage.

#40 Section: Vaccines and Clinical aspects of RSV

Title: Induction of specific Th1 immunity in mice using recombinant adenoviruses carried fusion protein gene

Authors: I-Hwa Chen, Yana Chen, Fabin Ebenezer Chitra, Charles Sia, Yen-Hung Chow.

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Abstract: Respiratory Syncytia virus (RSV) has since been recognized as the leading cause of lower respiratory infections in children and increasingly documented as an important pathogen in elderly. Previous studies indicate that immunizing with formalin-inactivated RSV evokes a Th-2-cell response which has been suggested to play a role in enhanced disease in vaccines upon live virus exposure. Therefore, a safe and efficacious RSV vaccine should induce a Th-1 cell response associated with the production of cytotoxic T-lymphocytes (CTL). The present study attempts to develop such a vaccine by expressing structural proteins in an adenoviral delivery vector in order to protect the elderly against RSV infection. The RSV Fusion protein, F, has been selected as the vaccine immunogen since it can induce RSV-neutralizing antibody as well as cellular immunity. The full length (F0) and transmembrane-truncated (ΔTM) genes with different functional domains were amplified by PCR and cloned into the *adenovirus type 5* system. The individual adeno-constructs were evaluated as delivery vectors and tested for gene expression by SDS-PAGE/Western blot *in vitro*. In order to evaluate the effect of recombinant adenoviruses-F (rAd-F) vectors on the activation of immune responses in two different strains of mice, Balb/c and C57BL/6 mice intranasally (prime) and intramuscularly (boost) two times infected with rAd-Fs, assessment of neutralizing anti-RSV antibodies, Th-1/-2 cytokines and subsets of T-cells were performed, The results from our studies show that rAd-F0 and rAd-F0ΔTM induces systemic antibody (IgG) responses which neutralized RSV. There are no difference of humoral responses between Balb/c and C57BL/6 mice. The IgG1/IgG2a ratios indicated a Th1-mediated antibody response. Similar to the antibody responses, we observed that rAd-Fs induced a both Th1 (IFN-r/IL-2) and Th2 (IL-4) cytokine profiles with a Th1-toward immunity. We also examined the population of CD4+/CD25+/FoxP3+ Treg cells and CD4+/CCR5+/CD62L+ memory T cells in RSV- or rAd-F-infected mice and found that rAd-F decreased the percentage of Treg cells in spleen (1-2%) compared to RSV-infected groups (~4%). In contrast, increased the percentage of memory T cells were found in rAd-F-infected mice. These data suggest that rAd-F may induce specific immunity, which is Th1-biased responses and may be a potential vaccine candidate against RSV.

#41 Development of two real-time RT-PCR for the detection of human respiratory syncytial virus A and B and human metapneumovirus A and B.

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The objective was to develop two real-time RT-PCR for the detection and the typing of the human respiratory syncytial virus (hRSV) and human metapneumovirus (hMPV).

Material and Methods

Nasal samples from children hospitalized included 41 hMPV-positive and 91 hMPV-negative specimens, and 44 hRSV-positive and 91 hRSV-negative samples. Viral detection was carried out by a routine procedure. The two real-time RT-PCR were developped in collaboration with Argene (Varihles, France). Primers and probes are complementary to N gene of hRSV A and B, and M gene of hMPV A and B. Reactions were carried out in the Smart-Cycler II (Instrumentation Laboratory, Cepheid). HRSV and hMPV assays were done in separate tubes using the same protocol. Viral RNA were also used in an in-house multiplex RT-PCR.

Results

The tests were able to detect plasmid dilutions with reproductible detection of 100 copies per reaction in 100% of cases and detection of 10 copies per reaction in 30% of cases, for both hRSV and hMPV. No cross-reactivity was observed with extracts from other viruses or bacteria.

All the 41 hMPV DFA-positive and 91 hMPV DFA-negative samples were confirmed positive in both the real-time RT-PCR and the multiplex RT-PCR. Three additional samples were found positive using the hMPV real-time RT-PCR. Two of them were also detected in cell culture, and in the multiplex RT-PCR. The 91 hMPV DFA-negative samples included 38 specimens containing another respiratory virus. The 44 hMPV-positive specimens in the real-time RT-PCR included 15 hMPV group A and 29 hMPV group B. Seven of them were negative in the multiplex RT-PCR.

All the 44 hRSV DFA-positive and 91 hRSV DFA-negative samples were confirmed positive in the real-time RT-PCR and one was missed by the multiplex RT-PCR. Seven additional samples were found positive using the hRSV real-time RT-PCR, six of them being missed by the multiplex RT-PCR. The 91 hRSV-negative samples in the DFA included 42 specimens containing another virus. The 52 hRSV-positive specimens in the real-time RT-PCR included 49 hRSV group A and 3 hRSV group B. Eight of them were negative in the multiplex RT-PCR.

Conclusion.

The two real-time RT-PCR were sensitive and specific assays for the detection and the typing of hMPV and hRSV in nasal samples. They detected 3 (2.3%) more hMPV-positive and 7 (5.1%) more hRSV-positive samples than the DFA.

#42 Section: Vaccines and clinical aspects of RSV

Title: Reduced intracellular TLR4 in neutrophils in Bronchoalveolar lavage fluid (BAL) and blood of infants with severe RSV Bronchiolitis.

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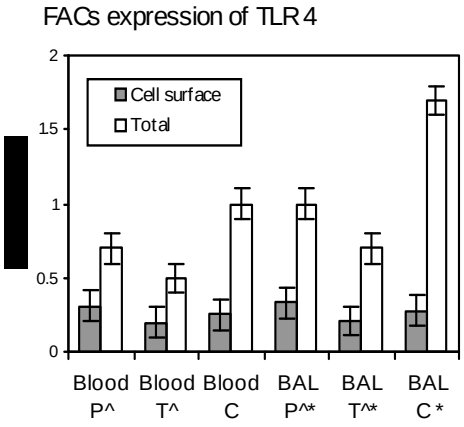
Abstract: The massive inflammatory infiltrate in the lungs of infants with severe RSV Bronchiolitis is mainly neutrophils (>85%), little is known about the role they play in the innate immune response to RSV infection Toll like receptors (TLR) are expressed on the cell surface (TLR 1,2 ,4,5,6) or within the endosome (TLR 7,8,9,10) of neutrophils. The role of neutrophil TLRs in the pathogenesis of RSV disease is unclear.

Methods: Following informed consent, blood and BAL samples were obtained from 20 (9 term (T)and 11 preterm(P)) RSV positive infants with severe Bronchiolitis and 7 controls(C), aged less than 18 months, immediately after intubation. Neutrophils, enriched using sedimentation gradient techniques, were analysed by flow cytometry (FACs). Each FACs assay was tripled labelled with CD16 PE, Ethidium Monoazide Bromide and TLR 2, 4 7, 8, 9 antibodies. All results were standardised into mean Antigen Binding Capacities.(ABC)



Results. The samples contained >95% neutrophils. All neutrophils expressed TLRs 2,4,7,8,9. The expression of TLR 7, 8, 9 in blood and BAL was 10 times less than TLR 2 and 4. Diagram shows that there were significant differences ($P<0.05$) in the intracellular expression of TLR 4 between Blood and BAL (*) and between controls and cases (^). There were no significant changes in the expression of TLR 2 between cases/controls, Blood/BAL and when comparing cell surface with total expression. There was no significant difference in the intracellular expression of TLR 7, 8, 9 in the neutrophils of the blood or the cases and the controls

Conclusions: There is significantly less intracellular TLR 4 in the BAL and Blood of infants with severe RSV disease. This may explain why they are predisposed to severe infection.

#43 Section: Vaccines and Clinical aspects of RSV

Title: Modulation of the Th2-biased immune response to RSV induced by DNA vaccines delivered by gene gun

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Abstract: Gene gun delivery of DNA vaccines induces a strongly biased Th2 response. As a result, BALB/c mice vaccinated with plasmids encoding the F or G proteins of RSV develop pulmonary eosinophilia following RSV challenge. In order to determine whether the Th2 response generated by gene gun vaccination with either pF or pG could be modulated to a more balanced response, the effects of CpG oligonucleotides (ODN), a plasmid expressing the heat-labile enterotoxin of *Escherichia coli* (pLT) or topical imiquimod (IMQ) were assessed in BALB/c mice. Vaccination with pF or pG and CpG ODN increased the IgG2a:IgG1 ratio of RSV-specific antibodies in a proportion of mice compared with those given pF or pG with GpC ODN, but did not have a significant effect on induction of pulmonary eosinophilia, 6 days after RSV challenge. Vaccination with pF and pLT or pG and pLT induced a significant increase in the ratio of RSV-specific IgG2a:IgG1 antibodies in all mice vaccinated with pF and pLT, but not in those vaccinated with pG and pLT. Although there was a decrease in the number of IL-5 producing cells in the lungs of mice vaccinated with pG and pLT or pF and pLT, 6 days after RSV challenge, compared with mice vaccinated with pF or pG and pCon, pLT did not have a significant effect of on induction of pulmonary eosinophilia following RSV challenge. IMQ treatment also induced a significant increase in the ratio of RSV-specific IgG2a:IgG1 antibodies. However, IMQ treatment resulted in a significant increase in the numbers of IL-2, IL-5, IL-4 and IFNγ producing cells in the lungs and did not reduce the extent of pulmonary eosinophila, 6 days after RSV challenge. Although IMQ was the most effective adjuvant for inducing a balanced RSV-specific IgG2a:IgG1 antibody response, further work is required to find a way of modulating the immune response induced by gene gun vaccination with pF or pG in order to prevent the induction of pulmonary eosinophilia following RSV challenge.

#44 Section: Vaccines and Clinical Aspects of RSV

Title: A dose-ranging study of a subunit Respiratory Syncytial Virus subtype A vaccine(V) adjuvanted(adj) with aluminum phosphate((RSV-A-alum) in adults ≥65 years of age(y)

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Introduction In high-risk persons (≥65 y, immunocompromised, underlying cardiopulmonary disease) RSV is associated with similar morbidity to influenza virus. A randomized, dose-ranging, placebo-controlled, single-blind, phase II trial of RSV A-alum containing subunit antigens F, G and M was conducted to determine its safety and immunogenicity in older persons, and its effect on influenza vaccine (InfV) immunogenicity.

Methods: 561 adults ≥65 years of age at 5 Canadian sites were randomized to one intramuscular injection (IM) of either 100, 50 or 25 μgs RSV-A-alum or 100 μgs non-adj RSV-A, or alum-placebo. All subjects were offered InfV on day (d) 32. RSV serology (neutralization assay by plaque reduction method (NA) v. RSV A and B; ELISA titres v RSV F, G, M) was done days 0, 32, 60, 180 and 360. Influenza serology (serum hemagglutinin inhibition (HAI) reciprocal titers) was done d32, d60, d120. Adverse events (AE) were assessed d0-8, d120, 180 and 360 and severe/serious AE throughout the study.

Results: Immunization was well tolerated, with a similar reactogenicity to alum-placebo and infV. Five serious AE were unrelated to V. Only the 100 μg non-adj RSV V achieved ≥4 fold antibody rise in NA v RSV-A in ≥50% of subjects at d32. Dose-response was seen at d32 for adj vaccines. GMTs v RSV-A and B at all points were comparable in 100 μg adj and non-adj groups NA titres were maintained by >75% of these participants on d180. A d 32 ≥4-FAR or HI ≥40 to Influenza (A-H3N2) was seen in >74% of subjects; no difference was seen between groups.

Conclusions: A subunit RSV-A V was well tolerated in a large population ≥65 y and did not interfere with InfV immunogenicity. Both adj and non-adjRSV vaccine containing F, G, and M antigens at the 100 μg dose showed encouraging results with respect to antibody titers compared to placebo.

 # 45 Section: Vaccines and Clinical aspects of RSV

Title: Identification of the component responsible for the type III hypersensitivity reaction (HS III) induced by BBG2Na, a subunit Respiratory Syncytial Virus (RSV) vaccine

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BACKGROUND: RSV is a pneumovirus causing severe LRTI in young infants, individuals at risks and the elderly. BBG2Na is a chimeric protein comprising: BB an albumin-binding domain and G2Na the conserved domain of RSV G protein. BBG2Na/Adju-Phos was found to be immunogenic and safe in phase I and II clinical trials in adults. However, during the clinical phase III in the elderly, 3 out of 665 vaccinees developed unexpected purpura, a HS III reaction known to be mediated by immune complexes. Arthus reaction (AR) is the standard animal model for HS III. The aim of this study was to confirm the ability of BBG2Na to induce AR and to identify the component of BBG2Na responsible for the observed adverse events in rabbit model.

METHODS: To first validate this model we assessed proteins included in human vaccines for which AR have been reported, namely diphtheria (DT) and tetanus toxoids (TT). Groups of 10 rabbits received 4 weekly i.m. injections of antigen (100 mcg). Seroconversion was checked and the AR was triggered on day 35 by i.d. injection of antigen (100 or 1000 mcg). Haemorrhage and oedema were then scored 3 or 6 hrs after triggering. The evaluation of BBG2Na, BB and G2Na was separately performed in groups of rabbits (total number of 235).

RESULTS : DT and TT clearly induced an AR. In contrast to G2Na, BBG2Na and BB reproducibly induced a positive AR which was time- and dose-dependent. This suggests that high affinity of BB to human serum albumin may contribute to the observed reaction.

CONCLUSION : This study allowed us to discriminate proteins for their capacity to induce HS III. Furthermore, it clearly demonstrated that BB was responsible for the purpura observed in BBG2Na phase III clinical trial. In parallel to this study, immunogenicity and protective efficacy of G2Na were confirmed in rodents. G2Na is a safe RSV vaccine candidate.

#46 Section: Vaccines and Clinical Aspects of RSV

Title: Prostaglandin E2 (PGE2) is Protective in Human Viral Bronchiolitis

Authors: Jayson Luma[1], Karen Hintz[2], Robert C Welliver Sr[2]

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Text: Several studies have established the presence of cysteinyl leukotrienes (LT) in the airways of infants with viral bronchiolitis. In contrast, little is known about the presence and significance of other arachidonic acid metabolites in this condition. We measured LTB4, LTC4, LTE4, PGD2 and PGE2 in respiratory secretions of infants with bronchiolitis caused by infection with either respiratory syncytial virus (RSV) or parainfluenza virus (PIV), correlating measured quantities of each metabolite with simultaneous values of oxygen saturation.

PGD2 and PGE2 were the arachidonates present in the highest concentrations, followed by LTB4> LTC4 = LTE4. Measured quantities of LTB4 were highly correlated ($p<.001$) with all other mediators except PGE2. LTC4 and LTE4 were highly correlated with each other and with PGD2, but not with PGE2. PGD2 and PGE2 were not significantly correlated ($p = .14$).

Quantities of PGE2 were protective against severe disease, being directly correlated with oxygen saturation values ($p = .028$) and inversely with length of stay in hospital ($p = .039$). No other arachidonate bore any significant relationship to oxygenation or length of stay.

PGE2 appears to be synthesized separately from other arachidonates in human bronchiolitis. Quantities of PGE2 are associated with improved oxygenation and shorter hospital stays.

#47 Section: Vaccines and clinical aspects of RSV

Title: Intranasal immunization of mice with a formalin-inactivated bovine respiratory syncytial virus vaccine co-formulated with CpG oligodeoxynucleotides and polyphosphazenes results in enhanced protection

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Abstract: Respiratory syncytial virus (RSV) targets the mucosal surfaces of the respiratory tract, which suggests that both systemic and mucosal immune responses need to be induced to achieve optimal protection. A formalin-inactivated bovine RSV (FI-BRSV) vaccine was co-formulated with CpG oligodeoxynucleotides (ODN), non-CpG ODN, polyphosphazenes (PP), or CpG ODN and PP, and delivered intranasally to BALB/c mice. CpG ODNs are immune modulators that signal through Toll-like receptor-9 and PPs are thought to form non-covalent complexes when mixed with antigens and other adjuvants, increasing their stability and allowing for multimeric presentation. FI-BRSV formulated with CpG ODN and PP elicited both humoral and cell-mediated immunity, characterized by enhanced production of BRSV-specific serum IgG, as well as increased IFN-γ and decreased IL-5 production by *in vitro* re-stimulated splenocytes. These immune responses were stronger than those elicited by FI-BRSV formulated with either CpG ODN, non-CpG ODN or PP. The mice also produced increased BRSV-specific IgG and IgA in the lungs, so they developed mucosal immune responses. The most striking differences between FI-BRSV formulated with both CpG ODN and PP in comparison to formulations with CpG ODN, non-CpG ODN or PP individually were increased serum and mucosal IgG, and in particular mucosal IgA and virus neutralizing antibodies. Furthermore, FI-BRSV/CpG/PP was the only formulation that resulted in a significant reduction in viral replication upon BRSV challenge. These data suggest that co-formulation of CpG ODN and PP is promising as an approach to mucosal immunization.

#48 Section: Vaccines and Clinical Aspects of RSV

Title: RSV Vaccine Development Based on Human Papillomavirus Virus-Like Particles

Authors: Yoshihiko Murata[1], Edward E. Walsh[1], Ann R. Falsey[1], and Robert C. Rose[1,2]

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Abstract: RSV remains a predictable and significant cause of respiratory tract illness in persons of all ages, especially in infants and children. As a novel approach towards an RSV vaccine, we plan to utilize virus-like particles (VLPs) formed by human papillomavirus (HPV) L1 and L2 capsid proteins. These VLPs can function as a self-adjuvanting antigen delivery system that elicits cellular and humoral immune responses against heterologous antigens. To this end, we isolated a full-length F cDNA from a clinical RSV isolate (RGH strain). We identified four domains (1: aa 23-122; 2: 154-222; 3: 226-378; and 4, 379-523) of the F protein that lacked the transmembrane domain or stretches of hydrophobic amino acids and were sufficiently small (≤ 150 aa) to be fused to the C termini of HPV capsid proteins without adversely perturbing VLP formation. Domains 1, 3, and 4 bore previously identified epitopes for neutralizing antibodies and/or murine CTL responses. We then generated baculovirus stocks, each designed to express HPV L2N (truncated L2 protein; aa 1-237) bearing one of the RSV F domains at the C terminus. Using the baculovirus stocks, we expressed the HPV L1 protein together with each of the L2N-RSV F protein derivatives in *T.ni* insect cells. From cellular extracts, the resulting HPV/RSV chimeric VLPs (cVLPs) were purified using CsCl isopycnic ultracentrifugation and tested for purity, structural integrity, and presence of conformation-dependent neutralizing epitopes on HPV L1 protein. Our preliminary data suggest that the HPV/RSV cVLPs 1, 3 and 4 possess HPV L1 neutralization epitopes, contain L2N-RSV F fusion proteins of interest, and are of sufficient purity for use in mouse immunization experiments.

#49 Section: Vaccines and Clinical aspects of RSV

Title: Persistance of antibodies induced by a single injection of MEFG2Na a RSV Subunit vaccine encapsulated in microsphere in RSV primed BALB/c mice

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BACKGROUND: Respiratory syncytial virus (RSV) is a paramyxovirus causing severe LRTI in young infants, individuals at risks and the elderly. MEFG2Na is a recombinant protein comprising: MEF 3 aa residues and G2Na 101 aa of the central conserved domain of RSV G protein. In order to be in the elderly/adult situation, we evaluated in RSV primed BALB/c mice the persistance of RSV antibodies (Ab) induced by a single injection of MEFG2Na encapsulated in microsphere (/MS) or MEFG2Na/Alum (Al).

METHODS: Groups of BALB/c mice were primed i.n with RSV $10^5 TCID_{50}$ three weeks prior immunisation. After seroconversion confirmed, mice received a single im injection of MEFG2Na/MS, MEFG2Na/Al, empty MS, PBS/Al respectively. MEFG2Na and RSV ELISA were determined on day 7, day 14, day 21 .. up to day 148. Avidity of the induced Ab was also measured.

RESULTS : MEFG2Na/MS induced (i) primary RSV Ab responses similar to MEFG2Na/Al (ii) long term RSV Ab responses (day 148) significantly higher to MEFG2Na/Al. Furthermore, there was increase avidity of the induced Ab between day 48 and day 148.

CONCLUSION : in seropositive mice, a single injection of MEFG2Na/MS induced long lasting RSV Ab with affinity maturation. This result indicates that this type of formulation is potentially interesting vaccine candidate for the elderly or individuals at risks. Protective efficacy of this formulation is currently investigated in cotton rats.

#50 Section: Vaccines and Clinical Aspects of RSV

Title: Genetic analysis of a temperature-sensitive RSV vaccine candidate rA2cp248/404/1030ΔSH following in vitro replication at non-permissive temperatures

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Abstract: We performed *in vitro* expansion of a cold-passage (cp) temperature-sensitive (ts) RSV vaccine candidate, rA2cp248/404/1030ΔSH, in order to investigate mutations that may develop in response to growth at non-permissive temperatures. Wild type RSV can form plaques at 37°C and 32°C, whereas rA2cp248/404/1030ΔSH has a ≥3 log reduction in plaquing efficiency at 37°C compared to 32°C. In previous studies, virus shed from patients had a loss of the ts mutation 248 or 1030 (Karron, R. A., *et al*, 2005; Lin, Y-H. *et al*, 2006). However, it was noted that some shed viruses that had lost temperature sensitivity had maintained all the cp and ts markers, suggesting the presence of unidentified suppressor mutations. In our study, we passaged rA2cp248/404/1030ΔSH at non-permissive temperatures of 35°C and 37°C. Several ts partial revertants were obtained. The majority had reversions at the 1030 mutation in L, but retained all other attenuating cp and ts mutations. One virus retained the entire set of cp and ts mutations, but had acquired 2 additional mutations in L and a truncation of G. Growth kinetics performed at 32°C indicated that the revertants grew similarly to rA2cp248/404/1030ΔSH at the permissive temperature. Plaquing efficiency performed at 32°C, 35 °C, 36 °C, 37 °C, 38 °C and 39°C indicated that each of the revertants had partial, but not complete, loss of temperature sensitivity. Selective pressure of growth at physiologically relevant temperatures can be used to anticipate reversions and mutations that can impact temperature sensitivity phenotype of shed vaccine virus in clinical trials.

#51 Section: Vaccines and clinical aspects of RSV

Title: Rescue and characterisation of a recombinant Sendai virus expressing respiratory syncytial virus F protein.

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Abstract: Respiratory syncytial virus (RSV), of which there are two subgroups (A and B), is a major cause of morbidity and mortality in infants, the elderly and immunocompromised individuals. Despite many decades of research, there are no safe and effective vaccines against RSV and no specific therapeutics. Sendai virus (SeV), a murine paramyxovirus that is closely related to human parainfluenza virus type 1 (hPIV-1) was previously shown to have potential as a classical Jennerian vaccine against hPIV-1. We hypothesised, therefore, that expression of RSV antigens from a recombinant SeV might provide protection against both hPIV-1 and RSV. To address this hypothesis in relation to RSV, we rescued a rSeV expressing the RSV A Long strain fusion protein (rSeV/RSV F) and a control rSeV expressing enhanced green fluorescent protein (rSeV/EGFP). Both antigens were expressed from an extra-numeral transcription unit inserted into the same region of the SeV genome. RSV F was chosen because of its known capacity to induce protective immunity against both RSV subgroups. Efficient expression of RSV F was confirmed by immunofluorescence using a FITC-conjugated RSV F-specific monoclonal antibody. Functionality of the expressed RSV F protein was confirmed in a fusion assay on LLC-MK2 cells in the absence of acetylated trypsin, without which the SeV F protein is non-functional. To determine the vaccine potential of rSeV/RSV F against RSV, groups of BALB/c mice were inoculate intra-nasally (IN) with rSeV/RSV F, rSeV/EGFP or PBS. Three weeks later they were bled and challenged with RSV A strain A2 IN. Strong RSV antibody responses were induced in all rSeV/RSV F-inoculated animals, while controls demonstrated background levels. Furthermore, sterilising lung protection was evident in all rSeV/RSV F-immunised mice, in contrast to control animals, where high RSV titres were detected. These data demonstrate that a rSeV efficiently expressing a functional RSV F protein has potential as an RSV vaccine.

#52 Vaccines and Clinical aspects of RSV

Differential diagnosis of RSV-like acute lower respiratory tract infection in HIV positive, negative or exposed children in South Africa.

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Respiratory syncytial virus (RSV) is the most frequently detected virus in both HIV positive and negative children with acute lower respiratory tract infection (ALRI). Standard diagnostic tests for respiratory viruses include direct immunofluorescence assays (IF) which detect RSV, PIV1,2,3, Adenovirus, Influenza A and B and CMV and a rapid antigen detection assay for RSV. Although virus culture is considered the gold standard, the clinical relevance is limited by the long delay to diagnosis and culture difficulties. Between 30-60% of suspected respiratory infections remain uncharacterised. At least 6 new viruses associated with ALRI have been discovered since 2001 which may account for some of the unidentifiable cases. Improved sensitivity of molecular diagnostic techniques also lead to increased detection of viruses traditionally associated with upper respiratory tract infections in ALRI cases. The epidemiological importance of many of these viruses has not yet been determined. Increased disease burden following RSV and human metapneumovirus infection in HIV infected children has been demonstrated but the importance of none of the new viruses in this group is known. To address these issues a multiplex realtime PCR assay with hybridisation probes was developed that detects all the traditional viruses as well as hMPV, Boca virus; Coronavirus NL63, HKU1, OC43 and 229E in four reactions. As part of an epidemiological investigation of the new viruses in HIV positive and negative children in South Africa, multiplex PCR screening for these viruses as well as newly discovered polyomavirusses WU and KI a.w.a Rhinovirus was carried out. The study group consisted of 270 children with ALRI that tested negative to all viruses included in the conventional IF assay, 30 that were positive by IF and 30 from a control group without respiratory infections. Of these children 35% were either HIV infected or exposed. In addition, discrepancies in RSV IF and rapid tests lead to the sequencing of specific RSV strains, mostly from HIV positive children. Changes in the N-and G-proteins were detected that may suggest the emergence of altered strains in immunocompromised patients. These data contribute to our understanding of the epidemiological importance of respiratory viruses in HIV positive and negative children in Africa.

#53 Section: Vaccines and Clinical aspects of RSV

Title: Human Respiratory Syncytial Virus (HRSV) population dynamics in patients through time.

Authors: Mariana Viegas[1], Alberto Maffey[2] and Alicia S. Mistchenko[1]

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Abstract: The mechanisms of HRSV evolution during the course of natural infection and virus evasion from the immune response have not been completely elucidated. It is known that RNA viruses, such as RSV, are thought to exist in nature as distributions of genetic related variants known as quasispecies. The evolutionary plasticity of such distributions could account for the notorious ability of RNA viruses to evade host immunity. The genetic variability of HRSV during the course of infections at different times was analyzed. Pairs of nasopharyngeal aspirates (NPA) were taken from patients who had persistently respiratory symptoms (between 13 and 41 days). The ectodomain of the G-protein gene was amplified directly from NPA and cloned in the Pgem-t vector. Ten clones from each sample were sequenced and analyzed. They revealed an important genetic variability, with an average number of nucleotide differences per site ranging from 0.0123 to 0.0385. Most substitutions were non-synonymous and were situated in the two hypervariable regions. The phylogenetic analysis showed a remarkable heterogeneity in the genetic variants, especially, in those that belonged to a patient who had a cellular immune response altered. The first sample of that patient presented a more stable viral population, while the second one, 41 days after, showed a complex swarm of genetic variants even with two genotypes at the same time. The amino acids differences between clones were 2.3% and 7.2% for the first and second NPA respectively, showing a variability increase through time. The implications of these findings regarding virus evolution and clinical aspects of these patients will be discussed.

 # 54 Section Clinical and Vaccines

Title:Molecular Epidemiological Analysis of a Nosocomial outbreak of Respiratory Syncytial Virus associated pneumonia in a Kangaroo Mother Care Unit in South Africa

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Abstract
Background. Respiratory syncytial virus (RSV) may cause severe lower respiratory tract disease in premature infants. Prolonged viral shedding has been reported in patients with underlying immunosuppressive disorders, such as HIV-1 infection. During March to May 2006, 23 preterm pediatric patients developed nosocomial pneumonia in a secondary hospital in the Gauteng Province of South Africa due to RSV infection. The patients were identified using routine diagnostic testing. All had been admitted with their mothers to a Kangaroo Mother Care (KMC) ward from birth – a low care unit for the management of stable low birth weight infants. The HIV-1 sero-prevalence among the mothers to these infants was 50%, translating to a 50% perinatal exposure.
Methods. A Multiplex nested RT PCR was used to subtype RSV positive nasopharyngeal aspirates. Sequencing and phylogenetic analysis of part of the G-protein gene was used for molecular epidemiological analysis of the outbreak.
Results. Twenty of the 23 RSV positive specimens could be PCR amplified and sequenced. The Subtype A, GA5 genotype was identified in 14 specimens and the BA genotype, a new subtype B genotype not previously recognized in South Africa, in seven. One patient had an infection with both genotypes. Phylogenetic analysis demonstrated eight separate introductions. Two of the strains identified in this outbreak were circulating in a general pediatric ward during the preceding month.
Conclusions Prolonged asymptomatic RSV shedding by HIV-1 positive mothers and inadequate infection control measures by health care providers could potentially increase the risk of RSV infection in KMC wards.

#55 Section: Vaccines and Clinical aspects of RSV

Title

A highly attenuated recombinant human respiratory syncytial virus lacking the G protein confers long time protection against RSV infection in cotton rats.

Authors: M.N. Widjojoatmodjo[1], J. Boes[1], P.J. Roholl[2] and W. Luytjes[1],
[1] Department of Vaccine Research, Netherlands Vaccine Institute, Bilthoven, The Netherlands; [2] Microscopy Consultancy, Weesp, The Netherlands

Abstract:

Live attenuated RSV vaccines have seen difficulties in achieving the correct balance between safety and immunogenicity. A vaccine based on a recombinant RSV virus in which the single G gene was deleted was considered to be too attenuated because it was highly attenuated in the respiratory tract of mice. The cotton rat (Sigmodon hispidus) is more permissive for RSV and the infection model mimics much better the pathology observed in humans vaccinated with FI-RSV. Therefore, we studied infectivity and protectively of a recombinant RSV strain (rRSV-X) lacking G in the cotton rat. rRSV-X could replicate in the cotton rat lungs, but there was no detectable replication of a rRSV-X lacking G.

Standard intranasal infection models use a relative large volume which allows the vaccine virus to reach the lungs. Live attenuated intranasally administered RSV vaccines for infants should not reach the lungs. Therefore, we have set up an infection model using a low vaccine volume which infects only the NALT. Cotton rats were vaccinated on days 0 and 21, and challenged on day 36, 71, or 142. Animals were sacrificed 5 days post challenge. Both rRSV-X and rRSV-X lacking G prevented challenge virus replication in the lungs up to day 76. Although no virus could be detected, mild inflammatory changes were detected starting at day 75 for rRSV-X and at day 41 for rRSV-X lacking G. No elevated levels of eosinophils, one of the hall-marks of enhanced disease as is seen with FI-RSV vaccination, was detected at any time point examined.

In a separate experiment, it was shown that even a single dose of rRSV-X or rRSV-X lacking G conferred protection up to day 75 against viral replication. Vaccination with β-propiolactone inactivated rRSV-X lacking G did not induce protection. Thus, live virus is required for the induction of protection in cotton rats and the attachment glycoprotein G seems to be dispensable. Hence, RSV lacking G represents a promising live vaccine candidate.

 # 56 Section: Vaccines and Clinical aspects of RSV

Title: Down-regulation of IL4Rα using antisense oligonucleotides alleviates long-term pulmonary dysfunction induced by neonatal respiratory syncytial virus infection

Authors: Dahui You[1, 2], Michael Ripple[2], Andrew Sewell[1] and Stephania A. Cormier[1]

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Abstract: Respiratory syncytial virus (RSV) is the most common cause of upper and lower respiratory tract infections in infants. Infants who develop RSV-induced bronchiolitis are more likely to develop subsequent wheeze and/or asthma as children and adolescents. Currently, there is no vaccine or efficient antiviral treatment available for RSV. We have created a mouse model which mimics the pathophysiological changes produced by RSV in infants. In this model, neonatal infection with RSV induces long-term airways dysfunction and inflammation. Furthermore, our data and that of others suggest that the age of initial infection is critically important in determining secondary immune responses to RSV. Neonatal exposure to RSV resulted in elevated levels of IL-4 and IL-13 in the lung and the levels of IL-13 were further increased in mice upon secondary infection. IL-4 and IL-13 are central mediators of Th2 immune responses and play a major role, not only, in pulmonary inflammation and mucus production, but also, in regulating the development of Th2 cells. **We hypothesized that inhibiting IL-4 and IL-13 signaling using antisense oligonucleotides directed to IL4Rα (IL4Rα-ASO) prior to and after initial neonatal infection would prevent inflammation and subsequent airways dysfunction.** Seven day old BALB/c mice were inoculated intranasally with RSV; a cohort of mice was treated with IL4Rα-ASO. IL4Rα-ASO was administered intranasally at a dose of 100 μg/kg once daily for two days before through two days after RSV infection. Once mature, these animals exhibited improved lung function (i.e., decreased airways resistance and increased compliance) compared to mice infected with RSV as neonates and not treated with IL4Rα-ASO. This improvement in lung function correlated with decreases in RSV-induced pulmonary inflammation, mucus production and IL-13 levels in bronchoalveolar lavage fluid. These data suggest that employing IL4Rα-ASO as a vaccine strategy could prevent RSV-induced bronchiolitis and the development of subsequent wheeze and/or asthma associated with neonatal RSV infection.

#57 Section: Immunology

RSV-specific IFNg ELIspots as a Biomarker of T Cell Immune Function.

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Background: Biomarkers to assess T cell immune function have not been developed for potential clinical use. Respiratory Syncytial Virus (RSV) is a secondary infection in all adults since infection is universal by the age of 3 years and memory T cell responses have been demonstrated in many human peripheral blood mononuclear cells (PBMC). Unlike other viruses, RSV does not undergo major antigenic variation, and the predominant IFNg response with RSV stimulation is due to CD4 T cells and much less from CD8 T cells and NK cells. Therefore, our hypothesis is that RSV-specific IFNg response is potentially a good biomarker of T cell immune function.

Methods: We enrolled healthy adult subjects between the ages of 18 - 65 years old during the summer and fall of 2006 to avoid RSV season which occurs between November and March of each year in Rochester, New York. Fresh human peripheral blood mononuclear cells (PBMC) were activated using live RSV antigen (MOI >1.0). We then measured memory RSV-specific IFNg produced by CD4 T cells using Elispot from whole PBMCs of each patient.

Results: All adult subjects had RSV-specific IFNg responses by the Elispot assay. Of the 52 healthy subjects and 49 immunocompromised Human Immunodeficiency Virus (HIV) subjects, RSV-specific IFNg responses range from 93 to 563 (mean ± SD 241 ± 116) and from 13 to 713 (154 ± 146) spots /million PBMC respectively. HIV subjects with CD4<200 had RSV-specific IFNg responses range 13 to 321 (54 ± 66) spots million PBMCs.

Conclusion: All healthy adult subjects have memory RSV-specific IFNg responses by the Elispot assay. RSV-specific IFNg responses <150 spots/million PBMC may serve as a good biomarker of T cell immune function.

 # 58 Section: Immunology-Innate and Adaptive

Title: The Development of RSV CD8+ T Cell Epitope Hierarchy is Regulated by Competition during Antigen Presentation as Evidenced by Vaccination Strategies

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Abstract: Understanding the basis for the establishment of CD8+ T cell epitope hierarchy and the induction of co-dominant responses may have important ramifications for designing vaccine strategies for viruses like HIV-1 or RSV. In the murine model of RSV, dominant epitopes have been defined in viral M and M2 proteins in H-2^b and H-2^d, respectively. In the CB6/F1 H-$2^{d/b}$ hybrid mouse, the M2 epitope is dominant over the M epitope which provides a unique model to study the basis for CD8+ T cell epitope hierarchy. First, we assessed whether competition for APC processing machinery during vaccination could impact subsequent responses post challenge by immunization with a recombinant adenovirus serotype 5 (rAd5) construct where both epitopes were expressed from the same gene. Next, we asked whether local competition during vaccination for access to APCs could impact the resultant post challenge hierarchy through immunization with either the M2 or M epitopes expressed from different genes and administered at separate injection sites. Data from tetramer staining of infected lung lymphocytes obtained post-challenge revealed the greatest increase in M-specific responses at days 7 and 10 whenever the competition between immunodominant M2 for APCs was eliminated. This increase suggests that co-dominance between epitopes can be improved by immunizing with rAd5 expressing individual epitopes. Furthermore, a greater proportion of independently induced M-specific lymphocytes secreted IFN-γ, TNF-α, and IL-2 indicative of an improved functional profile. Based on these results, we believe that the development of CD8+ T cell epitope hierarchy is in part determined within the APC during antigen processing and presentation, and this may be important for optimizing cellular immune responses induced by vaccines.

 # 59 Section: Immunology – Innate and Adaptive

Title: Human Metapneumovirus Perturbation of IFN-γ-Induced Gene Transactivation

Authors: Stephanie A. Campbell, Darrell L. Dinwiddie, Reed Taylor, Albert P. Senft, and Kevin S. Harrod[1]

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Abstract: Human metapneumovirus (hMPV) is a recently discovered Paramyxovirus closely related to RSV. Like RSV, hMPV infection is a significant cause of childhood respiratory illnesses such as wheezing, bronchiolitis, and pneumonia. Despite similarities in clinical presentation, hMPV and RSV share low genetic homology. In particular, hMPV lacks the NS1 and NS2 genes of RSV that are known to impair host innate immune functions. Although hMPV lacks these immunomodulatory genes, mouse models suggest that hMPV can develop persistent infections of the lung. Herein, we report that hMPV infection inhibits interferon (IFN)-γ-activated promoter gene expression in both A549 cells and macrophages. However, hMPV does not prevent IFN-γ-mediated phosphorylation or nuclear translocation of STAT1 in these cell types, suggesting a unique mechanism of IFN-γ regulation. Interestingly, the splice variant STAT1-β was detected in the nuclear fraction of hMPV-infected cells following IFN-γ treatment, while STAT1-β remained cytoplasmic in uninfected IFN-γ-treated cells. STAT1-β, which differs from STAT1-α by the absence of the CREB-binding protein-interacting domain, has been reported to block STAT1-mediated transcriptional activation in a dominant negative fashion. Our findings suggest that STAT1 DNA binding may be disrupted during hMPV infection, and this disruption may be mediated by increased levels of the dominant negative STAT1-β isoform. These findings suggest a novel mechanism by which hMPV may regulate IFN-γ signaling and has broad implications for immune function in viral host defense.

 # 60 Section: Immunology: Innate and Adaptive

Title: CD14 positive cells mediate the Respiratory Syncytial Virus induced pro-inflammatory effect on neutrophils.

Authors: Christopher M. Coleman[1], Lynsey Hobson[1], Karen Plant[1], Richmond Muimo[1], Moira K.B. Whyte[3] and Mark L. Everard[2].

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Abstract:

Neutrophils were isolated from citrated venous blood (from healthy adult volunteers) by a discontinuous Plasma-Percoll gradient and additionally purified by negative immunomagnetic bead selection. Freshly isolated neutrophils were challenged with A2-RSV or UV-inactivated virus at a multiplicity of infection (m.o.i.) of 0.5, 1, 2 or 10.

The effect of RSV on neutrophil survival was measured with Annexin V-PE /To-Pro-3 staining and by morphological changes, using Dif-Quick staining of cytocentrifuge slides. Both Flow Cytometry and cytospin analysis show prolonged neutrophil survival in the presence of A2-RSV. A2-RSV inhibited the apoptosis of neutrophils isolated by Percoll gradient separation in a dose-dependent manner over a 24 hour time-course. When neutrophils were Ultra-purified, inhibition of neutrophil apoptosis by RSV was restricted to just the highest virus concentration over the full time course and the m.o.i. of 2 at 8 hours post-infection. Depleting the Percoll gradient purified cell population of cells by positive immunomagnetic selection, using antibodies against the monocyte marker CD36, the T-cell marker CD3, the B cell marker CD19 and the Natural Killer Cell marker CD56 has no effect on the inhibition of apoptosis, whilst depletion using CD14 has the same effect as ultra-purification.

Cytokine Bead Array analysis was performed on media samples from Percoll and Ultra-purified cells challenged with A2-RSV at m.o.i. of 2 and 10 for 12 hours. Many pro-inflammatory cytokines and chemokines were up-regulated in the Percoll-pure cultures, but only the chemokine MIP-1β was also up-regulated in the Ultra-pure cultures.

These data suggest that RSV primarily affects neutrophil survival indirectly, possibly through a CD14 positive cell type, and may be MIP-1β dependent.

61 Section: Immunology

Analysis of the Human B Cell Repertoire Against Respiratory Syncytial Virus G Protein

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Respiratory syncytial virus (RSV) is a ubiqutous virus causing serious respiratory tract infections in the young, elderly and those in high-risk groups. Development of an effective vaccine has proven problematic with early efforts actually exacerbating morbidity. Today, the only effective intervention is Synagis™, a humanized monoclonal antibody against the RSV F protein that is useful as a prophylactic treatment. The RSV attachment (G) protein is significant in virus replication, and the G protein is linked to immune modulation. Antibodies to G protein are likely to reduce virus infection and have therapeutic potential. To explore this therapeutic mode, the B cell repertoire of 18 subjects with documented RSV infection was surveyed for reactivity to G protein from RSV A and B strains as well as F protein using the microscopic multiplexing technology, CellSpot™, developed at Trellis Bioscience. The highest numbers of circulating B cells with reactivity to any RSV protein occurred 4-8 weeks after infection and waned over time. Numbers of circulating RSV-specific B cells one year after infection were relatively low. Analysis revealed three specimens with B cells producing cross-reactive antibody to G protein from RSV A and B strains. B cells producing these antibodies were cloned by limiting dilution, and V_H and V_L regions amplified and cloned into a human IgG1 expression vector. The binding activity of these monoclonal antibodies was confirmed by ELISA on RSV lysates. The resulting fully human antibodies were selected *in vivo* to avoid off-target reactivity and therefore should represent ideal therapeutic candidates for clinical testing of the role of RSV G blockade as a post-infection therapeutic intervention.

 # 62 Section: Immunology – Innate and Adaptive

Title: Toll-like receptor 4 modulates the protective antibody response against respiratory syncytial virus through its interaction with the viral glycoproteins.

Authors: Silvina Coviello[1], Juan P. Batallé[1], M. Florencia Delgado[1], Andrea Pontoriero[2], Peter Collins[3], Vilma Savy[2] and Fernando P. Polack[1,4].

Affiliations: [1]Fundación INFANT and [2]INEI-ANLIS C. G. Malbran, Buenos Aires, Argentina, [3]NIAID, NIH, Bethesda, MD; and [4]Johns Hopkins University, Baltimore, MD.

Abstract: Toll-like receptor 4 (TLR4) is an important pattern recognition receptor that activates the immune system through interaction with structurally conserved molecules derived from microbes, including the respiratory syncytial virus (RSV) fusion (F) glycoprotein. RSV F is highly conserved and essential for virus viability. In addition, F is the main neutralizing antigen in RSV. We describe a critical interaction between TLR4 and the highly conserved F that increases the antibody response against the virus. Activation of TLR4 by RSV F increases neutralizing antibodies (shown against RSV G), promotes affinity maturation of the antibody response against RSV, and can also enhance the protective antibody response against other viruses, like influenza H_1N_1. Demonstration of a similar effect using other TLR4 agonists (i.e.: endotoxin) and lack of enhancement of the antibody response in TLR4-deficient mice confirmed the critical role of the pattern recognition receptor in this effect. The other neutralizing RSV glycoprotein (G), which inhibits TLR4 activation, negatively modulated antibody production. G inhibition is exerted through its highly conserved cysteine-rich region. Our findings highlight critical interactions between an important receptor in the innate immune system and highly conserved regions in the main RSV glycoproteins that, independent of epitope-specific responses, are essential for promoting protective antibodies against the virus.

 # 63 Section: Immunology – Innate and Adaptive

Title: Human Metapneumovirus Inhibits IFN-α Signaling Through Inhibition of the Phosphorylation of STAT1

Authors: Darrell L. Dinwiddie and Kevin S. Harrod

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Abstract:

Human metapneumovirus (hMPV) is closely related to respiratory syncytial virus (RSV) and is a major cause of lower and upper respiratory tract infections worldwide. Despite similarity in tropism and clinical presentation, hMPV lacks substantial genetic homology to RSV. One striking difference is the lack of obvious nonstructural (NS) proteins that function to inhibit IFN-mediated viral clearance. Yet, the ability of hMPV to establish a persistent infection in mice implies that hMPV may in indeed possess mechanisms to subvert host immunity. In lung epithelial cells, infection with hMPV prevented IFN-α-mediated transactivation of the interferon stimulated response element (ISRE) and upregulation of interferon stimulated genes (ISGs), indicating that hMPV is able to modulate IFN-mediated viral clearance mechanisms. In hMPV-infected lung epithelial cells, STAT1 phosphorylation and nuclear translocation was inhibited. The inhibitory effects of hMPV on STAT1 phosphorylation and translocation occurred in a dose-dependent manner and were abolished by UV-inactivation, suggesting STAT1 inhibition is dependent on viral gene expression and/or replicating virus. Phosphorylation of STAT2, Tyk2 or Jak1 by IFN-α, and the cell surface expression of the IFN-α receptor were unaltered by hMPV infection indicating that hMPV regulates IFN signaling at the level of STAT1 function. These findings demonstrate that hMPV can inhibit the type I interferon response, despite the lack of genes homologous to known immunomodulatory genes of other viruses and through a mechanism distinct from that of RSV.

#64 Section: Immunology – Innate and Adaptive

Title: Mechanism of STAT2 degradation by respiratory syncytial virus NS1 Protein.

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Abstract: Respiratory syncytial virus (RSV) is a major human pathogen, particularly of infants, the elderly and immunocompromised individuals. It is a member of the *Paramyxoviridae* family and has a linear single-stranded RNA genome encoding 11 proteins, 2 of which are nonstructural (NS1 and NS2). *In vitro* RSV was shown to specifically downregulate STAT2 protein expression, thus enabling the virus to evade the host type I interferon response. STAT2 degradation is mediated by the proteasome following infection and both the NS1 and NS2 proteins are implicated. Sequence alignment with prototypic proteins known to be components of ubiquitin ligase (E3) enzymes suggested that NS1 may have similar properties. Therefore, we investigated whether RSV NS proteins can assemble ubiquitin ligase (E3) enzymes to target STAT2 to the proteasome. We demonstrated that NS1 contains elongin C and cullin 2 binding consensus sequences and can interact with elongin C and cullin 2 *in vitro*; therefore, NS1 has the potential to act as an E3 ligase component. In contrast, NS2 did not demonstrate any such properties. Both NS1 and NS2 were independently capable of inducing STAT2degradation, although most efficient STAT2 degradation occurred when both NS proteins were co-expressed. By knocking down expression of specific endogenous E3 ligase components using small interfering RNA, NS1 and 2, or RSV-induced STAT2, degradation is prevented. These results indicate that E3 ligase activity is crucial for the ability of RSV to degrade STAT2. These data may provide the basis for therapeutic intervention against RSV and/or logically designed live attenuated RSV vaccines.

#65 Section: Immunology – Innate and Adaptive

Title: Differential Lung Dendritic Cell Recruitment in Respiratory Syncytial Virus and Human Metapneumovirus experimental infections.

Authors: Antonieta Guerrero-Plata[1], Deepthi Kolli[1], Antonella Casola[1,2,3] and Roberto P. Garofalo[1,2,3]

Affiliations: [1]Departments of Pediatrics, [2]Microbiology & Immunology, and [3]Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX 77555

Abstract: Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are two close related paramyxoviruses that induce similar symptoms in infected hosts. However, they do not induce the same response in all aspects of the immune system including innate immunity. Currently, there are not parallel studies regarding the recruitment of pulmonary dendritic cells (DC) in response to these two paramyxoviruses. In this work we characterize in detail the trafficking of DC subsets to the lung during RSV and hMPV infections in an experimental mouse model. Three subsets of pulmonary DC have been described in mice: conventional DC (cDC, $CD11c^{+}MHCII^{+}$), plasmacytoid DC (pDC, $CD11c^{int}B220^{+}Ly6C^{+}$), and the recently characterized IFN-producing killer cells (IKDC, $CD11c^{int}B220^{+}CD49b^{+}$). Mice were inoculated with RSV, hMPV or PBS (control). Recruitment of DC to the lung in inoculated mice was determined by flow cytometry at different time points. Our data indicate that both infections induced a peak of cDC recruitment ($>3x10^{6}$) by day 8 for RSV and by day 10 for hMPV. cDC remained elevated throughout the inflammatory and the resolution phases of infection ($1x10^{6}$) compared to control mice ($1x10^{5}$). RSV induced a peak of $8x10^{5}$ pDC recruited by day 3, while hMPV induced similar numbers but at day 8. pDC returned to basal levels ($1x10^{5}$) by day 6 (RSV) and by day 12 (hMPV). IKDC mirrored the pattern of migration of pDC but overall numbers of IKDC were smaller: peak for RSV was $5x10^{5}$ IKDC and $<4x10^{5}$ IKDC for hMPV. In conclusion, this study demonstrates that all three subsets of DC are differentially attracted to the lung in the course of RSV and hMPV infections, which in turn may differentially influence other aspects of the innate and/or adaptive immune response to these viruses.

66 Section:Immunology – Innate and Adaptive

Title: **Virally delivered cytokines alter the immune response to future lung infections**

Authors:James Harker [1], Alexander Bukreyev [2], Peter L. Collins [2], Belinda Wang [1], John S. Tregoning [1] and Peter J. M. Openshaw [1].

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Abstract:Respiratory Syncytial Virus (RSV) is an important cause of infant morbidity and mortality worldwide, increasingly recognised to have a role in the development and exacerbation of chronic lung diseases. There is no effective vaccine and we reasoned that it might be possible to skew the immune system towards beneficial non-pathogenic responses by selectively priming protective T cell subsets. We therefore tested recombinant RSV (rRSV) candidates expressing prototypic murine Th1 (Interferon gamma) or Th2 (Interleukin-4) cytokines, with detailed monitoring of responses to subsequent infections with RSV or (as a control) influenza A. Although priming with either recombinant vector reduced viral load during RSV challenge, there was disease enhancement upon challenge in mice primed with rRSV/ IFN-γ characterised by weight loss and an enhanced pulmonary influx of RSV-specific $CD8^+$ T cells. By contrast, rRSV/IL-4 primed mice were protected against weight loss during secondary challenge, but showed airway eosinophilia. When rRSV/IL-4 primed mice were challenged with influenza, disease was attenuated but was again accompanied by marked airway eosinophilia. Thus, immunization directed towards enhancement of Th1 responses reduces viral load but is not necessarily protective against disease. Counter to expectation, Th2-biased responses were more beneficial but also influenced the pathological effects of heterologous viral challenge.

#67 Section: Immunology-Innate and Adaptive

Title: RSV Replication Is Attenuated by Counteracting Expression of the Suppressor of Cytokine Signaling (SOCS) Molecules

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Abstruct: Human respiratory syncytial virus (RSV) causes an annual epidemic of respiratory tract illness in infants and in elderly and high-risk adults. Mechanisms by which RSV antagonizes interferon (IFN)-mediated antiviral responses include inhibition of type I IFN messenger ribonucleic acid (mRNA) transcription and blocking signal transduction of Janus family kinase / signal transducer and activator of transcription (JAK/STAT) family members. The suppressor of cytokines signaling (SOCS) gene family utilizes a feedback loop to inhibit cytokine responses and block the activation of the JAK/STAT signaling pathway. We evaluated the potential of SOCS molecules to subvert the innate immune response to RSV infection. Among eight SOCS family genes examined, RSV infection up-regulated SOCS1, SOCS3, and cytokine-inducible SH2 protein (CIS) mRNA expression in HEp-2 cells. Suppression of SOCS1, SOCS3 and CIS by short interfering ribonucleic acid (siRNA) inhibited viral replication. Furthermore, inhibition of SOCS1, SOCS3, or CIS activated type I IFN signaling by inducing STAT1/2 phosphorylation, resulting in expression of IFN-inducible antiviral genes in RSV-infected cells. These results suggest that RSV infection escapes the innate antiviral response by inducing SOCS1, SOCS3 or CIS expression in epithelial cells, and that the antiviral effect of each siRNA for the SOCS genes is mediated by activating expression of STAT1/2 and IFN-inducible genes.

#68 Section: Immunology- Innate and Adaptive

A role for transcellular IgG transport in limiting growth of respiratory syncytial virus (RSV) in respiratory epithelium.

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Background: IgG and IgA are both present in human upper respiratory secretions. IgA transport into and through epithelial cells is dependent on the polymeric Ig receptor. The assumption has been that IgG in nasal secretions is a transudate from serum. However, recently a receptor, FcRn, responsible for placental IgG transport was identified in respiratory epithelial cells. The detection of FcRn receptor in the respiratory epithelium suggests a more dynamic IgG balance with the potential for IgG transport across or into epithelial cells.
Methods: Primary adenoid epithelial cells (HAE) are grown as monolayers on a collagen matrix or in a Transwell insert to confluency and to where resistance across the insert exceeds 300 ohms.cm2, These HAE model systems have been used to examine the effect of immunoglobulin on RSV replication. The transwell insert can test the effectiveness of antiviral IgG applied to either basolateral or apical sides.
Results: In early efforts to grow HAE IVIG was added for 5 days to limit endogenous adenovirus replication. In spite of no residual RSV neutralizing antibody in the supernatant RSV growth in IVIG exposed cells was diminished by 2 logs when compared with cells without exposure to IVIG. IVIG inhibition of RSV was not seen with adenoid fibroblasts derived from the same tissue as HAE or HEp2 cells. Anti-RSV neutralizing monoclonal antibodies did not significantly protect polarized cells from apical RSV infection when applied to the basolateral surface. However, when such monoclonals were applied to HAE monolayers followed by extensive washing to remove any free antibody there was marked inhibition of RSV growth. This inhibition was not seen in HEp-2 cells. Additionally IgG could be demonstrated to be have entered or become associated with the cells by Western Blot. By immunohistochemistry HAE do express FcRn.
Conclusions HAE provide model systems for further exploration of the dynamics of immunoglobulin transcytosis across epithelial cells and mechanisms of viral inhibition by antibody at the epithelial surface or within epithelial cells. Further studies of IgG and IgA antibody transcytosis on inhibition of RSV growth are in progress. The complex interactions between IgG and FcRn, including pH dependence, remain to be elucidated. Never-the-less the studies suggest that topical application of RSV monoclonal antibodies may have a role in the treatment of RSV infection.

#69 Immunology Section

RSV G Glycoprotein Binds DC-SIGN/R, but This Interaction is Not Required for RSV Infection

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It has been demonstrated that RSV binding and infection calcium-dependent in both epithelial cells and in primary human dendritic cells (DCs), suggesting a role for C-type lectins. Algorithms developed during studies of the interactions between dendritic cell surface immunoglobulin (DC-SIGN) and HIV-1 predicted potential interactions of DC-SIGN and RSV glycoproteins. Using BIAcore technology, we examined the binding of DC-SIGN/R to purified RSV G. Interactions between RSV G and DC-SIGN/R were demonstrated, although relative affinity of the binding was approximately 10-fold less than seen with HIV-1 gp120. Additionally, the binding of DC-SIGN/R to RSV G was inhibited by the presence of EDTA or of mannans. Two approaches were taken to examine the role for DC-SIGN in RSV infection of DCs. Pretreatment of DCs with neutralizing anti-DC-SIGN/R antibodies did not inhibit RSV infection. Similarly, infection of Raji or K562 cells stably transfected with DC-SIGN/R did not facilitate infection. In contrast to the BIAcore binding studies, preincubation of DCs or HEp-2 cells with increasing concentrations of mannans did not reduce RSV infection. These data demonstrate that, while RSV G binds DC-SIGN/R, this binding is not required for productive RSV infection. The interaction between DC-SIGN/R and RSV G may have implications for RSV pathogenesis. Additionally, given that calcium is required for RSV infection, these data may suggest that low affinity interactions between RSV F and G glycoproteins and one or more C-type lectins may be required for RSV binding and infection. Studies to address these questions are underway.

#70 Section: Immunology – Innate and Adaptive

Title: Functional Maturation of the Human Antibody Response to RSV

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Abstract: Infant antibodies induced by viruses exhibit poor functional activity compared to those of adults. The human B cell response to respiratory syncytial virus (RSV) is dominated by use of the V_H3 gene family in both adults and infants, but only the adult sequences are highly mutated. Using a well-described neutralizing human monoclonal antibody to RSV, Fab19, we investigated the kinetic, structural, and functional advantage conferred by individual naturally occurring somatic mutations in the variable region of the antibody heavy chain (V_H). By comparing the fully affinity-matured Fab19 with Fab19-variants containing V_H germline amino acid residues, enhanced binding was achieved through naturally occurring somatic mutations in the third complementary determining region (HCDR3) that conferred a markedly faster on-rate and a desirable increase in antiviral neutralizing activity. Conversely, somatic mutations in the HCDR1 and HCDR2 regions did not significantly enhance antigen binding or antiviral activity. We observed a close correlation between the measured antibody affinity and antiviral activity, where an increase in antibody affinity resulted in an enhancement of antiviral activity. Moreover, a correlation was present between the association rate of antibody to viral antigen and antiviral activity, but a similar correlation was not observed with a change in the dissociation rate and antiviral activity. Structural studies of the predicted antigen-antibody complexes revealed the HCDR3 loop interacts with the antigenic site A surface loop of the RSV F protein, previously shown to be the epitope for this antibody. These structure-function studies suggest a molecular basis for the poor quality of antibodies made in infancy following viral infection or immunization. Our findings have the potential to lead to the rational design of novel vaccines that overcome the molecular barriers to generating an effective antibody response during infancy.

 # 71 Section: Immunology-Innate and adaptive

Title: Role of pulmonary macrophages in innate immune response to Respiratory Syncytial Virus (RSV) and human Metapneumovirus (hMPV)

Authors: Deepthi Kolli[1], Antonieta Guerrero-Plata[1], Chao Hong[1], Antonella Casola[1,2] and Roberto P Garofalo[1,2].

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Abstract: The lung macrophage population consists of alveolar macrophages residing at the luminal surface of alveoli and interstitial macrophages present within the parenchymal lung interstitium. The involvement of lung macrophages in innate immune responses to RSV or hMPV infections remains elusive. In this study we depleted lung macrophages in BALB/c mice by intranasal instillation of dichloro methylene bisphosphonate (Cl_2MBP) liposomes and examined lung inflammation and viral replication, along with cytokines and interferon (IFN) production following infection with RSV A2 or hMPV (can 9783). Macrophage depletion in RSV-infected mice caused a significant increase in lung viral replication ($P<0.01$) and lung inflammation ($14 \pm 1.54\%$ vs $40 \pm 8.8\%$, $P<0.05$) compared to undepleted infected mice. Surprisingly, in the case of hMPV infection a significant reduction in viral replication ($P<0.01$) was observed in macrophage-depleted mice compared to undepleted controls. Despite this opposite effect on viral replication, macrophage depletion resulted in a dramatic impairment in IFN-α and β production in both RSV and hMPV infection (~ 92-98% and 65-85% reduction in BAL IFN levels compared to undepleted mice for RSV and hMPV, respectively). Depletion of macrophages also caused suppression of RSV or hMPV-induced proinflammatory cytokines TNF-α and IL-6, chemokines RANTES and MCP-1, but increased secretion of IL-12 p40, IL-1α, IL-1β, G-CSF and IL-17. Flow cytometric analysis of lung revealed that macrophage depletion caused an increase in dendritic cell ($CD11c^{hi}MHCII^{hi}$) infiltration into the airways compared to undepleted mice after infection (both RSV and hMPV). In conclusion, our study demonstrates for the first time that in the course of RSV infection lung macrophages play an opposite role in controlling viral replication compared to hMPV infection, yet this is the major cell population responsible for the production of IFN type I and proinflammatory cytokines in response to both viruses.

72 Section: Immunology – Innate and Adaptive

Title: FcγR's are involved in antigen presentation during secondary RSV-specific $CD8^+$ T-cell responses.

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Abstract: Antigen-antibody (IgG) complexes can be internalized via Fcγ Receptors (FcγR) on antigen presenting cells, resulting in Ag processing, and presentation of peptides to $CD4^+$ T cells and cross-presentation to $CD8^+$ T-cells. In previous research we characterized different $H\text{-}2^b$ restricted epitopes derived from RSV proteins M (matrix), F (fusion), NP (nucleoprotein) and G (attachment glycoprotein). We observed a different hierarchy in the recognition of these epitopes by $CD8^+$ T cells during primary and secondary RSV infections in C57BL/6 mice. In the present study we evaluated a possible role of the humoral response during secondary T cell responses against RSV, comparing $CD8^+$ T cell responses in C57BL/6 WT and FcR common-γ chain K.O. and FcRn K.O. mice. We found that during secondary infections G protein specific $CD8^+$ T cell responses were significantly diminished, while the response against the dominant matrix epitope was not affected. Our results show the in vivo relevance of the FcR mediated cross presentation pathway during viral infection.

 # 73 Section Immunology

RSV-specific F and G Antibody Secreting Cells in Peripheral Blood during Acute Infection and from Long-lived Plasma Cells of Healthy Human Bone Marrow.

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Background: Low serum neutralizing antibody levels have been found to be a risk factor for infection and severe disease in adults. All adult subjects have evidence of RSV neutralizing antibody since infection is universal by age 3 years and repeat infections occur throughout adulthood. Despite similar pre-infection RSV antibody levels in the young and older subjects, older subjects have greater rises in RSV antibody levels after infection. RSV-specific F and G short-lived ASC frequencies in blood may best reflect the increase in antibody levels, and the antigen-specific long-lived plasma cell may best reflect the maintenance antibody levels. We demonstrate the ability to measure both human RSV-specific short- and long-lived ASC.

Methods: Healthy adult subjects were challenged with RSV A2 strain and peripheral blood mononuclear cells (PBMC) were obtained on day 0, 2, 4, 6, 8, 10, 12, 14, 21, & 28. Bone marrow (BM) was obtained from healthy young adults and long-lived plasma cells were isolated by positive CD138 selection. RSV-F-protein-specific, RSV-G-protein-specific, and total IgG antibody secreting cell (ASC) Elispots were performed directly ex vivo from PBMC or BM plasma cells.

Results: All subjects tested have ASC Elispot responses to the RSV F protein in the blood & bone marrow ex vivo. Many but not all subjects had ASC Elispot responses to the RSV G protein in the bone marrow ex vivo. BM plasma cell frequencies of RSV F protein and G protein were 0.5-8.2/1000 and 0-8.8/1000 of total IgG secreting cells respectively.

Conclusion: RSV-specific-F and -G protein antibody secreting cells can be detected directly ex vivo from peripheral blood during acute RSV infection and in bone marrow of healthy human non-RSV infected subjects.

#74 Section: Immunology – Innate and Adaptive

Title: Secondary RSV Infection: Balancing Clearance and Immunopathology

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Abstract: Natural infection with respiratory syncytial virus (RSV) confers incomplete immunity. However, there is considerable evidence that an anamnestic response to RSV is generated in both humans and animal models. In humans, reinfections are common throughout life, but most are not serious unless accompanied by other concommittant disease. We have chosen to use a secondary infection model to characterize the anamnestic response to RSV in a murine model with the ultimate goal on delineating protective versus pathologic immune responses to subsequent RSV infection.

Balb/c mice were infected with ~10^5 Line 19 strain of RSV and allowed to recover for 8-10 weeks. Primary infection in this model results in airway hyperreactivity, mucus hypersecretion, and a predominantly Th2 type response. RSV primed mice then received a second intratracheal challenge of virus, and the response was compared to controls receiving only a primary challenge.

Relative to the primary, the secondary response resulted in enhanced viral clearance and attenuated weight loss. However, secondary mice were not significantly protected from virus-induced AHR or mucus hypersecretion. The secondary response to RSV was associated with a dramatic increases in the early (day 2) production of chemokines CXCL9, CXCL10, and CCL2 in the lungs. A striking increase in plasmacytoid dendritic cells ($4.5 \pm 0.6 \times 10^5$ versus $1.4 \pm 0.3 \times 10^5$) was observed at day 2, with a comparatively smaller increase in myeloid dendritic cells ($23.8 \pm 2.1\ 10^5$ versus $16.4 \pm 2.1 \times 10^5$). Similar total numbers of CD4+ and CD8+ T cells were present in the lungs of both groups at day 2, but significantly higher in the lungs of secondary mice at day 8. Greater than 5-fold (21.9×10^5 vs 4×10^5) more RSV-specific CD8+ T cells (as assessed via $M_{82\text{-}90}$ tetramer) were present in the lungs at this time point. At day 8, secondary mice had attenuated IL-13.

The secondary response to RSV displayed enhanced T cell responses and viral clearance, but did not result in protection from immunopathology, as assessed. Secondary responses were characterized by early chemokine production, increased dendritic cell recruitment, and altered T cell responses. Ongoing studies will investigate the contribution of these factors to viral clearance versus host immunopathology.

 # 75 Section: Immunology – Innate and Adaptive

Title: Identification of Two Novel Murine CD4+ T Cell Epitopes in RSV M and M2 protein

Authors: Jie Liu[1], Teresa Johnson[1], Tracy Ruckwardt[1], Kathryn Bonaparte[1], John Nicewonger[1], Man Chen[1] and Barney Graham[1,2]

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Abstract: CD4+ T cells play an important role in RSV pathogenesis. Not only have aberrant Th2 responses been linked to the RSV vaccine-enhanced illness and to allergic inflammation and airway hyperresponsiveness, but also to diminished CD8+ T cell responses. In the murine model of RSV, we found that both immunization and primary infection can lead to phenotypic changes in CD4+ T cells associated with functional maturation. However, there are only two reported CD4+ T cell epitopes for RSV in the murine system. To better define the role of CD4+ T cells in direct immunopathology and in the modulation of CD8+ T cell responses, we identified new CD4+ T cell epitopes in the M and M2 proteins using 15-mer peptides overlapping by 11. We found both M and M2 proteins contain distinct CD4 epitopes. Peptides 53, 54, and 69 stimulated CD4+ T cells from M and M2–immunized or RSV–challenged CB6 hybrid mice to produce both IL-2 and IFN-γ. Both epitopes are restricted by I–Ab, and interestingly, the relative expression of IL-2 and IFN-γ is different for each peptide. In vitro, CD4 T cells responding to these epitopes can modulate the CD8+ T cell response to their respective M and M2 epitopes by affecting CD8+ T cell cytokine production. We are currently defining the optimal sequence for each epitope and are actively characterizing additional features of the cytokine secretion profile and functionality of these cells. Understanding the role of CD4+ T cells for the induction of optimal CD8+ T cell responses, and how to control CD4+ T cell functions through immunization will be important for the development of vector-based vaccines. Having well defined MHC class II-restricted epitopes will make that work possible.

#76 Section: Immunology – Innate and Adaptive

Title: Differentiation and immune function of RSV infected dendritic cells

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Abstract: We have studied the interaction between both human and mouse DCs with RSV and the subsequent initiation of the immunological responses. RSV is capable of infecting human DCs and activation/maturation is comparable to influenza and cytokine matured DCs. However RSV infection greatly impairs proliferation of both naïve and memory $CD4^+$ and $CD8^+$ T cells. It appeared that a soluble factor produced by RSV infected DCs was responsible for the reduced proliferation of T-cells. In contrast to the effects on proliferation, the different routes of antigen presentation were unaffected in RSV infected DCs. To determine whether RSV also influences the function of DCs in vivo, we studied the migration of murine DCs during an RSV infection. After i.n. RSV infection, there was a rapid migration of $CD11c^+$ DCs from the lungs to the lung draining lymph nodes. We showed the presence of viral RNA in the migrating $CD11c^+/CD8\alpha^-$ cell population. Despite the rapid influx of RSV positive DCs into the lymph nodes, effective T cell stimulatory potential was acquired relatively late after the peak of migration. Different DC populations in the lymph nodes presented viral antigen to T cells and stimulated IFN-γ production. We are currently studying the efficacy of the different DC subsets to induce naïve T cell activation.

#77 Section: Immunology – Innate and Adaptive

Analysis of gene expression in human epithelial cells stimulated by Interferon-α isoforms and TLR agonists

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Infection by respiratory syncytial virus (RSV) has been documented to activate cellular and cytokine responses in various cell types in animal and human models. The Toll-like receptor (TLR) and interferon (IFN) pathways play significant roles in the cellular response to RSV, either directly due to RSV F-protein/TLR interactions, or in the course of host defense. Typically, IFN-□ is considered as a single entity rather than as a family of 12 highly homologous genes (two of which have allelic variants) with differential biological activity. Reports of differential expression of IFN-□ isoforms *in vitro* in response to viral and bacterial pathogens suggest that each of these isoforms elicits unique cellular responses that are biologically and clinically relevant. To that end, we have treated A549 cells *in vitro* with various purified IFN-□ isoforms, and measured expression of IFN response genes by qRT-PCR (Taqman).
We first compared treatment of A549 cells with IFNα1 and IFNα10 at 100 U/ml each. They induced expression values for *IRF7* (an interferon-response gene) of 13.7 ±1.6 and 21.6 ±2.0 fold over control, respectively. To address the influence of TLRs on IFNα-mediated signaling, we pretreated cells with the TLR7 agonist imiquimod (10 μM) and the TLR3 agonist Poly I:C (10 μg/ml) and found that they had no effect on *IRF7* expression induced by either of the IFN□ isoforms. Similarly, Poly I:C pretreatment had no effect on expression of *MX1,* another IFN□ response gene. Experiments are underway to determine the effects of IFNα isoforms on genes expressed in response to TLR stimulation. The current paradigm of the interaction between innate and adaptive immunity strongly suggests that deciphering the RSV-induced signaling patterns through TLR molecules and subsequent host IFNα responses will uniquely contribute to our understanding of the pathogenesis of diseases induced by RSV.

#78 Section: Immunology – Innate and Adaptive

Title: Human Respiratory Syncytial Virus Nonstructural Proteins Modulate the Host SOCS Response to Infection in Murine Model of Infection

Authors: Lisa Moore and Ralph A. Tripp

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Abstract:

Respiratory syncytial virus (RSV) is an important respiratory pathogen causing lower respiratory tract illness in infants and children worldwide. Research suggests that RSV proteins may modulate the host response to infection to facilitate virus replication, e.g. RSV nonstructural (NS) proteins have been shown to be type 1 interferon (IFN) antagonists. It is known that suppressor of cytokine signaling (SOCS) molecules negatively regulate IFN expression. Here, we evaluate SOCS expression in a primary mouse lung epithelial cell line (MLE-15) following infection with wild type RSV or RSV mutant viruses lacking only the G gene or NS1 and NS2 genes. The results show that wild type RSV induces a SOCS-1 response and a reduction in type 1 IFN. However, infection with a RSV deletion mutant lacking both NS1 and NS2 genes results in a SOCS-3 response with enhanced interferon-α expression. In the BALB/c mouse lung, infection with RSV mutant viruses induce different patterns of SOCS and type I IFN expression compared to wild type RSV, suggesting that RSV proteins modulate the SOCS and type I IFN response possibly to facilitate RSV infection.

#79 Section: Immunology – Innate and Adaptive

Title: IL-17A is produced by OVA-specific CD4+ T cells with RSV infection during ongoing allergic inflammation

Authors: Dawn C. Newcomb, Martin Moore, Kasia Goleniewska, Weisong Zhou, R. Stokes Peebles, Jr.

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Respiratory syncytial virus (RSV) infections cause a large number of asthma exacerbations each year, and these infections are linked to increased airway responsiveness (AR) and heightened airway mucus production. In mice only infected with the A2 strain of RSV, we have not found increased AR; yet, mice infected with RSV during ongoing ovalbumin (OVA)-induced airway allergic inflammation (OVA/RSV) resulted in augmented AR that was strongly associated with lung IL-17A production. However, the antigen-specific T cell population responsible for lung IL-17A production has been undefined. Does allergic airway inflammation change the T cell response to RSV infection, so that RSV specific T cells make IL-17A, whereas in the absence of allergic inflammation RSV specific T cells predominantly make IFN-γ? Or, does RSV infection increase lung IL-17A production in OVA-specific T cells, whereas these same OVA-specific T cells produce very little IL-17A in the absence of RSV infection? Based on the pre-existing allergic inflammation and the presence of OVA-specific T cells at the time of infection, we hypothesized that RSV infection augments OVA-specific T cell production of IL-17A in the lungs of OVA/RSV mice. To test this hypothesis, we used intracellular cytokine staining in both wild-type and D011.10 transgenic mice which express an OVA specific T cell receptor that can be identified with the KJ126 antibody. Mice were sensitized with an I.P. injection of OVA/alum on day -16, and challenged with 1% OVA aerosol on day -2 through 5. Mice were infected with RSV on day 0 and lungs were harvested 6 days post infection, the peak of lung IL-17A expression. In wild-type mice, IL-17A was almost exclusively found in CD4+ T cells and not in CD8+ T cells. These IL-17A+, CD4+ T cells did not produce IFN-γ. In D011.10 mice, IL-17A production was from KJ126+, OVA-specific CD4+ T cells. These data suggest that allergen-specific CD4+ T cells, and not RSV-specific T cells, are the cell population responsible for IL-17A production. In addition, RSV infection augments the allergen-specific T cell production of IL-17A and therefore fundamentally changes the host immune response against an allergic stimulus.

#80 Section: **Immunology-Innate and Adaptive**

Title: **Differential Ability of RSV F- and M2-specific CD8 T Cells to Modulate Respiratory Syncytial Virus (RSV) Vaccine-Enhanced Disease**

Authors: **Matthew R. Olson[1], Steven M. Varga[1,2]**

Affiliations: [1] **Department of Microbiology,** [2] **Interdisciplinary Program in Immunology**, **University of Iowa, Iowa City, IA, 52242, USA**

Abstract: RSV infection is the leading cause of severe respiratory illness in young children. Children vaccinated with a formalin inactivated-RSV (FI-RSV) vaccine exhibited enhanced disease and pulmonary eosinophilia following a natural RSV infection. This disease can be mimicked in BALB/c mice by immunization with a recombinant vaccinia virus (vv) expressing the RSV attachment (G) protein containing a CD4 T cell epitope and no known CD8 T cell epitopes. Mice immunized with a 1:1 mixture of vvG and a recombinant vv expressing the RSV M2 CD8 T cell epitope (vvM2) do not develop pulmonary eosinophilia after RSV challenge. Interestingly, mice immunized with a 1:1 mixture of vvG and a vv expressing the RSV fusion (F) protein (vvF), still develop pulmonary eosinophilia despite mounting a robust F-specific CD8 T cell response similar in magnitude to that of the M2-specific CD8 T cell response in vvG+vvM2-immunized mice. Additionally, virus clearance from the lungs is virtually identical in vvG+vvF- and vvG+vvM2-immunized mice. These data suggest that F- and M2-specific CD8 T cells differ qualitatively in their ability to inhibit RSV vaccine-enhanced pulmonary eosinophilia.

#81 Section: Immunology-Innate and Adaptive

Title: Following type I interferon production during respiratory syncytial virus infection and re-infection *in vivo*

Authors: Lioubov M. Pletneva[1], Otto Haller[2], David D. Porter[1], Gregory A. Prince[1] and Jorge C. G. Blanco[1]

Affiliations: [1]Virion Systems Inc., 9610 Medical Center Drive, Suite 100, Rockville, Maryland 20850; [2]Abteilung Virologie, Institut für Medizinische Mikrobiologie und Hygiene, Universität Freiburg, D79008 Freiburg, Germany

Abstract: Respiratory syncytial virus (RSV) is the primary cause of bronchiolitis in young children. In general RSV is considered to be a poor inducer of type I (α/β) interferons (IFNs). Measurement of active type I IFN production during infections *in vivo* is demanding since multiple IFN subtypes with overlapping activities are produced. In contrast, Mx gene expression, which is tightly regulated by type I IFN, is easily determined. We therefore measured Mx expression as a reliable surrogate marker of type I IFN activity during RSV infection *in vivo* in the cotton rat model. We showed that the expression of Mx genes was dramatically augmented in lungs of infected animals in a dose- and virus strain-dependent manner. The expression of Mx genes in lungs was paralleled by their induction in nose and spleen, although in spleen no simultaneous virus gene expression was detected. Re-infection of RSV-immune animals leads to abortive virus replication in the lungs. Thus, type I IFN and Mx gene expression was triggered in re-infected animals even though virus could not be isolated from their lungs. Furthermore, we demonstrated that immunity to RSV wanes with time. Viral replication and Mx gene expression became more prominent with increasing intervals between primary infection and re-infection. Our results highlight the role of type I IFN in the modulation of the immune response to RSV.

#82 Section: Immunology – Innate and Adaptive

Title: The role of TNF in RSV disease in the mouse.

Authors: Philippa Pribul, James Harker, Deena Blumenkrantz, Debbie Lee, Jürgen Schwarze and Peter Openshaw.

Affiliations: Respiratory Medicine, Imperial College, St Mary's Campus, Paddington, London, W2 1PG, UK

Abstract: TNF has a well established pivotal role in the inflammatory cascade and has been implicated in many lung diseases, including RSV infection. Anti-TNF therapy is a successful treatment for chronic inflammatory conditions such as rheumatoid arthritis and has shown positive results in various pulmonary diseases including asthma.
Using a novel sub-clone of A2 strain RSV, incremental doses of intranasal virus caused increasing dose-related weight loss and overt illness in BALB/c mice. In RSV infected mice, depletion of TNF reduced weight loss, improved lung function, diminished dendritic cell (DC) maturation, attenuated RSV-specific CD8 responses and enhanced viral load.
The peak of detectable TNF in the lung lavage (BAL) of RSV infected mice is within the first 24 hours post infection. To investigate the possibility that macrophages are the major source of this TNF, mice were given clodronate liposomes by inhalation 3 days prior to infection with RSV. This procedure depleted alveolar macrophages and abolished the peak of TNF, IL-6, MIP1-α and IFN-α in the BAL after RSV. Moreover, depleted mice did not recruit or activate natural killer cells into the lungs and showed reduced CD8 T cell expansion after RSV infection. However, weight loss, lung function, DC maturation and RSV-specific serum antibody production were not affected.
We conclude that anti-TNF treatment is a potential therapy for RSV pathology but at the cost of increased viral load. We also show that although macrophages play a critical early role in cytokine and chemokine production, cell mediated immunopathology arises independently.

 # 83 Section: Immunology – Innate and Adaptive

Title: Regulatory T Cell Depletion Affects the Kinetics and Immunodominance of the CD8+T Cell Response following Respiratory Syncytial Virus Infection

Authors: Tracy J. Ruckwardt, Kathryn L. Bonaparte, Jie Liu, Man Chen, and Barney S. Graham

Affiliations: Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD

Abstract:
CD4+CD25+Foxp3+ natural regulatory T cells, in addition to controlling autoimmunity and anti-tumor immunity, are global regulators of adaptive immune responses. Anti-CD25 antibody (PC61) treatment prior to primary RSV infection can partially deplete these cells, and has several effects on the RSV-specific CD8+ response in the mouse model. Mediastinal lymph node and spleen epitope-specific CD8+ T cell responses are enhanced in Treg-depleted mice at all time points following infection, but T cell responses in lung show a strikingly different pattern with an early delay in the CD8+ T cell response in the setting of Treg-depletion, followed by enhancement of the response at later time points. The delay in the CD8+ T cell response correlates with both a delay in viral clearance and a delay in illness in Treg-depleted mice when compared to isotype-depleted controls. However, enhanced CD8+ T cell responses at later time points increase the severity of illness in depleted mice, and depleted mice were shown to have increased lung chemokine and cytokine levels 7 days post-infection. Interestingly, Treg depletion was shown to have a greater effect on the dominant M2 epitope response than on the subdominant M epitope response, indicating that regulatory T cells may modulate immunodominance disparities in the RSV-specific response following primary infection.

84 Section: Immunology – Innate and Adaptive

Title: Comparison of strain specific antibody responses during primary and secondary infections with respiratory syncytial virus

Authors: Paul D. Scott[1], Rachel Ochola[2], Charles Sande[2], Mwanajuma Ngama[2], Emelda A. Okiro[2], Graham F. Medley[1], D. James Nokes[1, 2], and Patricia A. Cane [3]

Affiliations: [1] Department of Biological Sciences, University of Warwick, UK; [2]Centre for Geographic Medicine Research – Coast, Kenya Medical Research Institute, Kilifi, Kenya; [3] Centre for Infections, Health Protection Agency, UK.

Abstract: Respiratory syncytial virus (RSV) repeatedly reinfects individuals: this may be partly due to the variability of the attachment (G) glycoprotein and changes in this protein have been shown to be under positive selection. Infants experiencing their primary infection show a genotype-specific antibody response with respect to the variable regions of the G protein. A prospective study of RSV infections in a birth cohort in rural Kenya identified infants experiencing repeat infections with RSV. The serum antibody responses of these infants were investigated with respect to their anti-RSV reactions in an enzyme-linked immunoabsorbent assay (ELISA) and the specificity of the response to the variable regions of the G protein by ELISA and immunoblotting using bacterially expressed polypeptides representative of the currently circulating strains of RSV. We confirm that the primary antibody response to the variable regions of the G protein is specific, but show that the response may become cross-reactive (at least within group A viruses) during persistent or secondary infections even when the secondary infection is of the same genotype as the initial infection. Also, some infants who did not mount a detectable antibody response to whole RSV antigens during their primary infection nevertheless showed genotype-specific responses to the G protein. In conclusion, the strain-specific nature of the serum antibody response to the variable regions of the G protein of RSV observed in primary infections is not maintained in subsequent reinfections and instead becomes cross-reactive.

 # 85 Section: Immunology-Innate and Adaptive

Title: Characterization of RSV F immune response induced by a chimeric bovine/human PIV3 vaccine candidate expressing RSV-F.

Authors: E.A. Stillman[1], C.L. Nelson[1], J. Guzzetta[1], M. MacPhail[1], R.R. Spaete[1], R.S. Tang[1]

Affiliations: [1]MedImmune, Inc., 297 N. Bernardo Ave, Mountain View, CA 94043

Abstract: A chimeric bovine/human PIV3 vector in which the RSV glycoprotein F is expressed from the second position and in which the PIV3 F and HN proteins are hPIV3-derived (MEDI-534) is currently being evaluated in Phase I clinical trials. Previously in hamsters, MEDI-534 has been shown to provide protection against challenge with RSV even though it induces neutralization titers that are lower than that induced by infection with wt RSV A2 (J.Virol 2003 77:10819). This current study was set up to further characterize the RSV F-specific immune response induced by MEDI-534. B-cell ELISPOT assays that quantify the RSV-F specific transient antibody secreting cells in hamsters, indicate that MEDI-534 induced a more robust F-specific response than wt RSV A2. Western blot analysis further confirmed that MEDI-534 induced an RSV-F-specific antibody response that was more robust than that observed in RSV infected animals. These results also indicated that the majority of the antibody response in RSV infected hamsters was directed against the G protein. T-cell ELISPOT assays indicated that MEDI-534 induced an F-specific CD8+ T cell response in mice that was reduced compared to wt RSV A2 but comparable to a highly attenuated RSV vaccine candidate. These data indicate that vectoring expression of RSV F from a PIV3 backbone, induces both a serological response as well as a CD8 T cell response against a major RSV antigen and thus may provide broad protection against RSV infection.

 # 86 Section: Immunology – Innate and Adaptive

Title: Influence of a Single Viral Epitope on T Cell Response and Disease after Infection of Mice with Respiratory Syncytial Virus

Authors: Simone Vallbracht[1], Birthe Jessen[1], Sonja Mrusek[1], Anselm Enders[1], Peter L. Collins[3], Stephan Ehl[1]* and Christine D. Krempl[2,4]*

Affiliation: [1]Center for Pediatrics and Adolescent Medicine and the [2]Department of Virology, Institute for Medical Microbiology and Hygiene, University Hospital Freiburg, Germany; [3]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, USA; [4]present address: Institute of Virology and Immunobiology, University of Würzburg, Germany
*These authors contributed equally to this work.

Abstract: Cytotoxic T cells (CTL) are important for virus clearance, but also contribute to immunopathology after infection of BALB/c mice with respiratory syncytial virus (RSV). The pulmonary immune response to RSV is dominated by a CTL population directed against the CTL epitope M2-1 82-90. Infection with a virus carrying a M2-1 N89A mutation introduced by reverse genetics failed to activate this immunodominant CTL population leading to a significant decrease in the overall antiviral CTL response. There was no compensatory increase in responses to the mutated neoepitope, to the subdominant epitope F 85-93 or to yet undefined minor epitopes in the N or the P protein. However, there was some increase in the response to the subdominant epitope M2-1 127-135 which is located in the same protein and presented by the same H-2K^d MHC molecule. Infection with the mutant virus reversed the oligoclonality of the T cell response elicited by the wild-type virus. These changes in the pattern and composition of the antiviral CTL response only slightly impaired virus clearance, but significantly reduced RSV induced weight loss. These data illustrate how T cell epitope mutations can influence the virus-host relationship and determine disease after an acute respiratory virus infection.

 # 87 Section: Pathogenesis II and Pulmonary Aspects of RSV

Title: IL-13 is Required for Respiratory Syncytial Virus Vaccine-Induced Pulmonary Eosinophilia

Authors: Elaine Castilow[1] and Steven M. Varga[1,2]

Affiliations: [1]Interdisciplinary Graduate Program in Immunology, [2]Department of Microbiology, University of Iowa, Iowa City, IA 52242

Abstract: Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract disease in children. Immunization of young children with a formalin-inactivated RSV vaccine led to enhanced morbidity and mortality upon natural RSV infection. Histological analysis of the lungs upon autopsy revealed pulmonary eosinophilia. BALB/c mice primed with a recombinant vaccinia virus (vv) expressing the attachment (G) protein of RSV develop pulmonary eosinophilia and vaccine-enhanced disease mimicking the response of the vaccinated children. Although immunization with the G protein elicits a mixed Th1 and Th2 response in these mice, the Th2 cells are known to be necessary for the induction of pulmonary eosinophilia. Here we have investigated the role of individual Th2 cytokines in mediating specific aspects of RSV vaccine-enhanced disease. We demonstrate that IL-13 and the IL-4 receptor α chain (IL-4Rα) are required for the development of eosinophilia in the bronchioalveolar lavage of vvG-primed mice challenged with RSV whereas IL-4 is not required. We find that in the absence of IL-4, IL-13 or IL-4Rα, inflammation, RSV G-specific T cell responses, viral clearance and weight loss are not altered. Histology performed on IL-13-deficient mice revealed a lack of eosinophils in the interstitium of the lung with no alteration in general inflammation. However, eosinophils were readily detected in the blood and bone marrow of IL-13-deficient mice. Our results suggest that IL-13 is necessary for the recruitment of eosinophils into the lung of vvG-primed mice upon RSV infection, and that distinct mechanisms control the pulmonary eosinophilia and the systemic symptoms associated with RSV vaccine-enhanced disease.

 # 88 Section: Pathogenesis

Title: Respiratory Syncytial Virus (RSV) infection in Hematopoietic Cell Transplant (HCT) Recipients: Impact of Immune Reconstitution

Authors: Yae-Jean Kim, MD[1], Janet A. Englund, MD[1,2], Katharine A. Kirby, MS[1], Ted Gooley, PhD[1] and Michael Boeckh, MD[1]

Affiliations: Fred Hutchinson Cancer Research Center[1] and Children's Hospital University of Washington[2], Seattle, WA.

Background Low absolute lymphocyte counts (ALCs) at the time of RSV upper respiratory tract infection (URI) have been associated with progression to lower respiratory tract infection (LRI) following HCT. Whether RSV LRI is, at least partially, due to an immune reconstitution syndrome is unknown. Therefore, we investigated the significance of changes in ALCs in HCT recipients with RSV URI.
Method Of 134 HCT recipients who underwent allogeneic HCT between Nov 1988 - May 2007 and had RSV URI, 34 patients (pts) progressed to LRI (progressors), whereas 100 pts had only URI (non-progressors). RSV disease progression was defined as documented LRI by radiographic criteria and RSV documentation by BAL or biopsy. Changes of paired ALCs were compared by Wilcoxon Signed Rank test. The delta ALC and slope of ALC changes were calculated in each group and mean values between groups were compared by Mann-Whitney test. Cox regression models were used to evaluate ALC changes as time-dependent risk factor for LRI and progression to LRI as a time-dependent risk factor for death.
Results: In the progressors, the median time for progression from URI to LRI was 7 days, with median ALC at URI vs. LRI of 137.5 vs.171 cells/mm^3. For non-progressors, the median ALC at URI (d0) and 7 days following URI (d7) were 295 and 379 cells/mm^3. Paired tests for ALC changes between URI and LRI in progressors and between d0 and d7 of URI in non-progressors did not show any significant differences (p=0.96 in progressors, p=0.13 in non-progressors). There were no significant differences between the groups in mean delta ALC (p=0.22) or mean slope of ALC (p=0.24). Also, ALC changes (delta ALC and slope) during 10 days after URI were modeled as time-dependent risk factors for LRI and did not show any significant association. Within 6 weeks from RSV URI, 23.5% (8/34) of progressors died compared to 15.3% (15/98, 2 lost to follow-up) of non-progressors. In time-dependent analysis, progressors had significantly greater hazard of death compared to non-progressors (HR=2.6, 95% CI=1.1-6.5, p=0.04).
Conclusions Immune reconstitution does not appear to be a significant factor in the progression from RSV URI to LRI during the first 100 days after HCT. The progression of RSV LRI more likely reflects invasive viral disease.

89 Section: Pathogenesis

Title: The Cytoplasmic Tail of G is a Novel Virulence Factor in Recombinant Pneumonia Virus of Mice

Authors: Christine D. Krempl[1,2], Anna Wnekowicz[1], Giw Nayebagha[1], Elaine W. Lamirande[3], Ursula J. Buchholz[3], and Peter L. Collins[3]

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Abstract: Pneumonia virus of mice (PVM) replicates very efficiently in mice and appears to be a natural mouse pathogen. Due to its virulence in a very convenient natural host it provides a reasonable model for studying the contribution of pneumovirus proteins in pathogenicity. The G proteins of RSV and PVM have been suggested to contribute to viral pathogenesis, but this had not been possible to study in a defined manner in a fully permissive host. We generated recombinant PVM bearing a deletion of the entire G gene (rPVM-GFP-ΔG) or expressing a G protein lacking its cytoplasmic tail (rPVM-GFP-Gs). Both G mutants replicated as efficiently *in vitro* as their recombinant parent but both were non-pathogenic in mice at doses that otherwise would be lethal. We could not detect replication of the ΔG mutant in mice, indicating that its attenuation is based on a severe reduction in virus load. In contrast, the Gs mutant appeared to replicate as efficiently in mice as its recombinant parent. Thus, the reduction in virulence associated with the Gs mutant could not be accounted for by a reduction in viral replication. These results identified the cytoplasmic tail of G as a virulence factor whose effect is not mediated solely by viral load. In addition to its intrinsic interest, a recombinant virus that replicates with wild type-like efficiency but does not cause disease defines optimal properties for vaccine development.

90 Section: Pathogenesis

Title: Human Respiratory Syncytial Virus persistent infection of epithelial cells: a model for virus-cell interactions

Authors: Isidoro Martínez[1], Luis Lombardía[2], Orlando Domínguez[2] , Blanca García-Barreno[1] and José A. Melero[1]

Affiliations: [1]Unidad de Biología Viral, Centro Nacional de Microbiología, Instituto de Salud Carlos III, and [2]Unidad de Genómica, Centro Nacional de Investigaciones Oncológicas, Instituto de Salud Carlos III. Madrid.

HEp-2 cells persistently infected with Human Respiratory Syncytial Virus (HRSV) were obtained as an outgrowth of cells surviving a HRSV lytic infection. These cells showed a characteristic cytophatic effect with large syncytia dominating the cell culture. However, small groups of non-syncytial cells were also observed and, as cells were passed, syncytia tend to become smaller and less numerous. Relatively high virus titers (between 10^6 and 10^7 PFU/ml) were detected in the culture supernatants of persistently infected cells. These supernatants interfered with the replication of wild-type virus, suggesting the presence of defective particles. However, no major changes in virus titers were observed during more than twenty cell passages. Western-Blotting of four viral proteins (G, F, NP and P) showed no apparent differences in the electrophoretic mobility between wild-type and persistent viruses. Indirect immunofluorescence revealed a cell heterogeneity in the persistently infected culture regarding viral expression. Cells expressing high amounts of virus antigens coexisted with cells that expressed low or not detectable antigens. Cloning of this cell population at different passages yield cells that grew slower than the HEp-2 cells and did not produce either infectious virus or viral antigens. These clones showed a partial restriction to wild-type virus replication characterized by a four-fold decrease in virus titer and a reduction in the number and size of syncytia compared to the original HEp-2 cells. Cellular mRNA expression was examined in persistently infected cells by cDNA microarrays. Differences in the expression of several cytokine and chemokine mRNAs was observed, including TNF-α and CCL2, when compared with lytically infected cells. These results show that persistent infections may be useful models for a better understanding of the interactions between HRSV and host cells.

 # 91 Section: Pathogenesis

Title: Molecular Basis of IL-13 and Mucus Induced by RSV Strain Line 19

Authors: Martin L. Moore[1], Michael H. Chi[1], Nicholas W. Lukacs[2], Peter L. Collins[3], Vasiliy V. Polosukhin[1], Kasia Goleniewska[1], Jamye O'Neal[1], and R. Stokes Peebles, Jr.[1]

Affiliations: [1]Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN; [2]Department of Pathology, University of Michigan Medican School, Ann Arbor, MI; [3]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Disease, Bethesda, MD

Abstract: Mucus overproduction is a hallmark of respiratory syncytial virus (RSV) disease and contributes to airway obstruction and respiratory failure. The cytokine IL-13 is a mediator of pulmonary mucus secretion. Mechanisms by which RSV induces IL-13 and mucus expression are unknown. We reported that infection of BALB/cJ mice with the line 19 strain, but not with the A2 strain of RSV results in lung IL-13, gob-5, and mucus expression [Lukacs et al. 2006. Am. J. Path. 169: 977-86]. Here, we sequenced the approximately 15.2 kb line 19 genome in order to define the region of line 19 responsible for mucus production. There are only eight nonsynonymous nucleotide differences between the line 19 and Long strains resulting in eight amino acid differences between line 19 and Long. These coding differences are in the F, NS1, L, and G genes. The Line 19 strain induced lung IL-13, gob-5, and mucus expression in BALB/cJ mice, whereas the Long strain did not. Thus, we identified eight candidate amino acids involved in RSV-induced IL-13 and mucus.

92 Section: Pathogenesis II and Pulmonary Aspects of RSV

Title: T Lymphocyte-Independent Antibody Responses in Primary RSV Infection

Authors: Jennifer Reed[1], Gary Sims[1], Timothy Welliver[1], LuAnn McKinney[1], Luis Avendano[2], Luis Velozo[3], Anthony J. Coyle[1], and Robert C. Welliver Sr[4].

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Abstract:

Respiratory syncytial virus (RSV) is the principal respiratory pathogen of early life. Little is known about induction of RSV antibody responses in infants. The nature of the immune response to RSV is not well understood, although primary infection is characterized by the absence of CD4 and CD8 antigen-positive cells in autopsy tissues, and by the absence of T-cell cytokines in nasopharyngeal secretions (NPS) of surviving infants. Nevertheless RSV specific antibodies are readily detectable following primary infection, suggesting T-independent B lymphocyte responses.
In autopsy tissues of fatal RSV bronchiolitis, CD20-positive B cells were abundantly present, and plasmacytes were visible. IgM, IgG and IgA antibody was identified in RSV-infected tissues in quantities far exceeding that in tissues from an uninfected infant. Type I interferon-inducible proteins such as MxA were strongly detected. B lymphocyte survival and proliferation factors BAFF and VIP were prominently expressed. In NPS from infants surviving RSV bronchiolitis, high titers of antibody to RSV in IgM, IgG and IgA isotypes were detected at an interval early after onset of illness. BAFF and VIP were detectable in all secretions and were associated with RSV specific and total antibody responses.
To determine whether Toll ligands associated with RSV infection might promote B cell activation, peripheral blood B cells were stimulated with anti-IgM and a TLR7 agonist in the presence of type I IFN and BAFF. TLR7 agonist or type I IFN alone did not induce Ig, but the combination did, and greater Ig synthesis and cytokine release were achieved when BAFF was added. Our data demonstrate that B lymphocytes, antibody, and B cell survival factors are all present during RSV bronchiolitis, while T cells are absent. While T cell education of B cells in the lymph node of neonates cannot be excluded, we propose that low affinity antibody responses following primary RSV infection may be T cell independent.

93 Section: Pathogenesis.

Title: Viral load in single and dual infection with hMPV and hRSV.

Authors: Malcolm G Semple[1], J Angela Booth[1,2], Bahram Ebrahimi[2]

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Abstract:

Background. In dual respiratory viral infections, hRSV involvement has been associated with an increase in severity of illness and a decreased IFN-gamma response (MG Semple 2005, JH Aberle 2004).

Hypotheses.

If increased severity of disease is a function of the presence of the second virus (synergistic pathology) then in infants with similar severity of bronchiolitis;

Dual infection with hRSV permits higher hMPV load than hMPV alone.

Dual infection with hMPV permits higher hRSV load than hRSV alone.

However, if risk of dual infection is a function of the infant's innate antiviral defence mechanisms, hRSV and hMPV load in infants with dual infection should correlate.

Method.

Infants (children <2 years old) admitted with a clinical diagnosis of bronchiolitis during the winter season 2004/5 were recruited with informed consent.

Nasopharyngeal aspirates (NPA) were collected within 24 hours of admission by a standard method.

hMPV L gene was detected and quantified by RT-qPCR in the NPA from 36 infants of which 24 were co-infected with hRSV. hRSV N gene was quantified by RT-qPCR in these samples 24 and in NPA from an additional 23 infants with similar disease severity who were hMPV negative. Median, quartiles and ranges are plotted for both data sets.

Comparisons were made of hMPV load in infants grouped by hRSV status and hRSV load in infants grouped by hMPV status. In infants with dual infection hMPV and hRSV load was compared, (scatter plot and Pearson's two tailed test with linear best fit).

Results.

Comparing single and dual infection;

- hRSV status has no significant effect upon hMPV load
- hMPV status has no significant effect upon hRSV load

In dual infection there was a significant correlation between hMPV and hRSV load (Pearson correlation 0.56, $p=0.02$) however scatter was large ($R^2=0.17$).

Conclusion.

This limited data does not support a mechanism of synergistic viral pathogenesis for dual hMPV/hRSV infection. Increased observation of dual infection in infants with severe bronchiolitis could be due to impaired innate antiviral defence conferring increased risk of dual infection and consequent increased viral cytopathic effects and immunopathogenesis.

94 Section: Immunology- Pathogenesis

Title: The role of TLR4 in severe respiratory syncytial virus lower respiratory tract disease.

Authors: María Elina Serra[1], Jacqui Marzec[3], Silvina Coviello[1], Maximiliano Salim[1,4], Andrea Rodríguez [1,5], Alejandro García [1,7], Daniela Bado [1,7], Ada Berenstein [8], Susana Siniawski [8], Miguel Bellabarba [1,5] , M. Florencia Delgado[1], Juan Pío Batallé[1], Ana Clara Monsalvo[1], Horacio Repetto [6], Steven R. Kleeberger[3] and Fernando P. Polack [1,2].

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Abstract: The pathogenesis of lower respiratory illness (LRI) elicited by respiratory syncytial virus (RSV) has been linked to inflammation. The RSV fusion (F) glycoprotein interacts with the pattern recognition receptor TLR4 and induces pro inflammatory cytokines. A highly conserved cysteine-rich region in the RSV attachment glycoprotein (G) antagonizes TLR-mediated inflammation. These interactions and the wide spectrum of severity in RSV LRI among infants with similar risk factors for severe disease suggest that genetic differences in TLR4 may affect the clinical presentation of RSV bronchiolitis. To explore the role of TLR4 in the severity of RSV bronchiolitis, we examined the association between two well-characterized TLR4 polymorphisms (SNPs), Asp299Gly and Thr399Ile, and severe disease (bronchiolitis requiring oxygen supplementation to maintain an O_2 sat ≥ 93%) in a multicenter case-control study. Postulated mechanisms of TLR4-mediated RSV illness were investigated, including modulation of virus clearance in nasal secretions, inflammatory cytokine production in the respiratory tract, and biasing of the Th adaptive immune response (peripheral blood mononuclear cells obtained 5-9 days after presentation and stimulated with F and G proteins). 785 previously healthy infants presenting their first LRI were enrolled. 444 infants with LRI were infected with RSV (194 mild vs. 270 severe), while 341 episodes of LRI were elicited by other viruses. TLR4 SNPs (examined by single-stranded conformation polymorphism) were associated with protection against severe RSV LRI [TLR4 SNPs in severe (5%) vs. mild (11%), *P*=0.03], but did not affect susceptibility to non-RSV severe LRI (*P*=NS). In our evaluation of infants from a low socioeconomic background, loss-of-function SNPs in TLR4 protected against severe RSV bronchiolitis.

95 Section: Pathogenesis II and pulmonary aspects of RSV

Title: Role of the SH protein in the pathogenesis of BRSV in calves

Authors: Geraldine Taylor[1], Sara Wyld[1] and Ursula Buchholz[2]

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Abstract: The role of the SH protein in the pathogenesis of RSV infection is not clear. When inoculated into mice, rHRSV∆SH resembled the parental wild-type virus in the efficiency of its replication in the lungs, whereas it replicated 10-fold less efficiently in the nasal turbinates [1]. However in chimpanzees, virus replication was similar to that of wild-type virus in the nose but was reduced 40-fold in tracheal lavage [2]. The SH protein of BRSV has only 38% amino acid identity with the HRSV SH protein and varies by up to 13% between different BRSV isolates. In order to investigate the role of the SH protein in the pathogenesis of BRSV, gnotobiotic calves were inoculated intranasally and intratracheally with 5 x 10^6 pfu of either rBRSV∆SH or parental wild-type BRSV. Replication of rBRSVΔSH was similar to or greater than that of wild-type rBRSV in the nasopharynx, and was not significantly reduced in bronchoalveolar lavage. However, rBRSVΔSH was isolated in low titres from lung samples obtained 6 days after infection and there was little or no gross pneumonic lesions at this time. In contrast, high titres of virus were isolated from lung samples of calves infected with wild-type rBRSV and calves developed gross pneumonic lesions. These observations suggest that although the SH protein does not influence virus replication in the upper airways, it is important in establishing lower respiratory tract infection.

[1] Bukreyev A., Whitehead S.S., Murphy B.R., Collins P.L., (1997) J. Virol. 71**:** 8973-8982.
[2] Whitehead S.S., Bukreyev A., Teng M.N., Firestone C.-Y., St. Claire M., Elkins W.R., Collins P.L., Murphy B.R., (1999) J. Virol. 73: 3438-3442.

 # 96 Section: Pathogenesis II and Pulmonary Aspects of RSV

Title: Respiratory syncytial virus strain A2 induces airway insensitivity to beta-adrenergic agonists in BALB/c mice

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Abstract: Beta-adrenergic agonists (β-agonists) are commonly used to treat respiratory syncytial virus (RSV) bronchiolitis, but are generally ineffective, for unknown reasons. In a BALB/c mouse model, we have previously shown that RSV strain A2 inhibits alveolar fluid clearance (AFC). In other lung injury models, impaired AFC can be restored with β-agonists, but following RSV infection, we found that β-agonists had no effect on AFC. Since bronchoalveolar epithelial insensitivity to β-agonists was induced in a paracrine fashion, the aim of the current study was to determine whether RSV also induces airway β-agonist insensitivity. Total lung resistance (R) was measured in anesthetized mice (6-8 per timepoint) undergoing mechanical ventilation on a computer-controlled piston ventilator (flexiVent®, Scireq). Data were analyzed using the single compartment model. Infection with RSV A2 did not induce airway hyperresponsiveness to increasing doses of the nebulized cholinergic agonist methacholine (MCH, 0.01, 0.1, 1, 10, and 20 mg/ml in isotonic saline) at any timepoint (2, 4, 6, or 8 d.p.i.). This finding is in agreement with previous studies using this viral strain. In separate groups of mice, doses of the β-agonist terbutaline (100 μM) were administered by nebulization prior to each dose of MCH (1, 10, and 20 mg/ml only). Prenebulization with terbutaline significantly attenuated (by 40-50%) bronchoconstrictive responses to 20 mg/ml MCH in uninfected mice and at 4-8 d.p.i. However, significant terbutaline insensitivity was noted at 2 d.p.i. – terbutaline only reduced the mean increase in R in response to 20 mg/ml MCH by 14% at this timepoint. This data, which is the first demonstration that infection with RSV results in airway insensitivity to β-agonists *in vivo*, suggests that desensitization of airway smooth muscle β-adrenergic receptors may underlie the modest utility of β-agonists as bronchodilators in therapy for acute RSV bronchiolitis.

97 Section: Therapeutics

Title: Decrease in pro-inflammatory mediators from Motavizumab-treated epithelial cells infected with Respiratory Syncytial Virus (RSV).

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Abstract: Respiratory syncytial virus (RSV) infection of epithelial cells is rapidly followed by the induction of a network of host responses including the release of cytokines, chemokines and low molecular weight mediators (leukotrienes and prostaglandins). Cytokines such as IL-6, IL-8 and TNF-α, and chemokines such as MIP-1α and RANTES have been shown to play a critical role in the inflammatory and immuno-regulatory aspects of RSV disease. In these studies, we tested whether motavizumab, a humanized $IgG_1\kappa$ monoclonal antibody (MAb) that is directed against the antigenic A site on the RSV F protein, could regulate epithelial cell immune responses to RSV. HEp-2 cells were infected with RSVA at multiplicity of infection of 1.0 and motavizumab, motavizumab Fab'2 or an irrelevant humanized $IgG_1\kappa$ MAb was added to a final concentration of 10μg/ml either 1 hour, 6 hours or 12 hours post infection. Supernatants were collected 6 and 24 hours after addition of the antibody and cytokines and chemokines were assayed. Cytokine, chemokine and viral gene expression were also assayed using real-time RT-PCR. Addition of motavizumab 1 hour post-infection to RSV-infected HEp-2 cells led to a significant decrease in gene expression of IL-6, IL-8, MIP-1α and RANTES and significant decrease in protein levels of IL-6, IL-8, TNF-α and IL-12p70. The decrease in the release of IL-6, IL-8, TNF-α and IL-12p70 was also seen when motavizumab was added 6 hours post-infection but not 12 hours post-infection. Addition of motavizumab Fab'2 gave results similar to those obtained with motavizumab indicating the lack of requirement for the Fc region. Addition of motavizumab either 1 hour or 12 hours post-infection led to an approximately 2 log reduction in RSV gene expression. These results suggest that therapeutic administration of motavizumab could lead to diminished pro-inflammatory epithelial cell immune responses and decreased viral replication.

 # 98 Section: Therapeutics

RSV therapy utilizing motavizumab, a second generation monoclonal antibody against F protein

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Respiratory Syncytial Virus (RSV) infection is the primary cause of pneumonia and bronchiolitis in young children which has been suggested to be a risk factor for the development of recurrent wheezing and long-term pulmonary dysfunction. Currently, Ribavirin is the only commercially available agent used to treat RSV infection, but its use is limited due to efficacy and toxicity concerns as well as the very long treatment regimen required for its delivery by aerosol inhalation. In this study, the therapeutic effect of motavizumab, an ultra-potent, humanized mAb against RSV F protein was evaluated in cotton rats. Groups of animals were infected with RSV A2, treated with motavizumab at different timepoints and sacrificed four days post infection. Efficacy was evaluated by measuring reduction of viral titers in lung and nasal tissue by plaque assay and qPCR. Viral replication was also assessed by staining of lung sections with a polyclonal anti-RSV antibody. Animals that had received motavizumab, showed significant decrease in lung viral titers, even at the late time point post infection. When lung sections were analysed for RSV, all groups showed positive staining for RSV of alveoli. However, lungs of animals treated early with motavizumab, showed reduced staining for RSV in the bronchioles, suggesting a preventive role of the antibody against viral spread. Our findings are relevant for the potential use of this antibody as a RSV therapeutic.

99 Section: Therapeutics

Title: Mechanism of action of TMC353121, a novel RSV fusion inhibitor with potent *in vitro* and *in vivo* activity.

Authors: Dirk Roymans[1], Tom Gevers[1], Peggy Geluykens[1], Heidi Szel[2], Peggy Janssens[2], Marcia Van Ginderen[1], Els Fransen[1], Rudy Willebrords[2], Eric Arnoult[3], Jean-François Bonfanti[3], Nick Verheyen[1], Barbara Van Kerckhoven[1], David Nauwelaers[1] and Koen Andries[2]

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Abstract:
Current treatment options for Respiratory Syncytial Virus (RSV) disease in all age populations offer limited efficacy, safety and convenience. Our RSV research program first led to the discovery of small molecule substituted benzimidazoles with subnanomolar *in vitro* anti-viral activity (1). However, most of these molecules displayed a prolonged elimination half-life from several tissues. Through a multidisciplinary approach, this chemical series was optimized, resulting in the selection of TMC353121, a compound with an optimal elimination half-life from lung ($T_{1/2}$ in lung = 25 h). TMC353121 showed a picomolar *in vitro* anti-viral activity (EC_{50} = 70 pg/ml) against RSV A and B subfamilies and different clinical isolates. In the Cotton rat model, TMC353121 demonstrated antiviral activity by intravenous, inhalation or oral route of administration. Time-of-addition experiments in a cellular model indicated that TMC353121 is active in the early phase of the RSV life cycle. *In vitro* selection of resistant mutants against TMC353121 further suggested that the compound binds to a hydrophobic region in the HR1-domain of the fusion protein. These results were confirmed by compound cross-linking experiments in WT and mutant HR1-derived peptides. Mutation of some of the hydrophobic region amino acid residues that seem to make electrostatic interactions with TMC353121 as suggested by molecular docking, indeed influenced the binding of the compound.
In conclusion, TMC353121 is a novel potent and specific RSV fusion inhibitor suitable for clinical evaluation.

(1) Andries et al, Antiviral Res **60**: 209-219, 2003.

 # 100 Therapeutics

RSV604 Blocks Replication of Respiratory Syncytial Virus in Organotypic Human Bronchial Epithelial Cell Cultures

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RSV604 {1-(2-fluoro-phenyl)-3-(2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4] diazepin-3-yl)-urea} is a low molecular weight inhibitor of respiratory syncytial virus (RSV) replication. The compound is currently in Phase I clinical testing. Based on mapping of resistant variants and recapitulation of the resistance phenotype by reverse genetics, the mechanism by which RSV604 inhibits RSV replication appears to be via effects on the viral nucleocapsid (N) protein. However, the specific molecular nature of this effect is not well understood. The compound has demonstrated activity in cell culture against both laboratory and clinical isolates of A and B serotypes of RSV, with EC_{50} values in the 0.4 to 1.0 μM range. In the presence of physiological concentrations of human serum albumin, the EC_{50} increases approximately 10-fold, consistent with its relatively high level of protein binding. RSV604 is not cytotoxic to actively dividing Hep2 cells at concentrations of >50 μM.

To assess the activity of RSV604 in a more relevant culture system, we have investigated its ability to inhibit RSV replication in differentiated primary human bronchial epithelial cell (HBEC) cultures. Primary bronchial epithelial cells grown in Costar Transwell inserts were cultured at an air-liquid interface for 14 days followed by infection with RSV strain Long by apical inoculation. Consistent with C^{14}-radiolabeled compound uptake studies, RSV604 added to the basolateral culture chamber demonstrated anti-RSV activity in this system with an EC_{50} value under 0.5 μM with no detectable cytotoxicity.

To confirm that viral genes are targeted by RSV604, we have selected multiple independent, altered susceptibility variants in Hep2 cell cultures. Currently, we are characterizing these variants for degree of resistance, cross-resistance to other inhibitors with known mechanisms of action, genetic changes and replication in HBEC cultures. The results of these studies will be discussed in the context of the proposed mechanism by which RSV604 inhibits viral replication, and will serve to characterize the variants for replication fitness in differentiated epithelial cell cultures.

101 Section: Viral structure, Entry, Replication and Cell biology

Title: Human respiratory syncytial virus P protein, only as tetramer, develops its activity on RNA viral synthesis.

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Abstract: To control respiratory infections caused for HRSV, a pneumovirus of the Paramyxoviridae family, specific antiviral compounds are useful tools. Ideal targets for these compounds are the ribonucleoproteins complexes (RNPs), the functional units for the transcription and replication processes. These processes are distinct of the viral metabolism. Thus, compounds able to interfering them must have no effect on cellular processes. The RNPs are composed by the viral RNA bound to the N protein forming the nucleocapsid and L, N, P and M2-1 proteins. P protein is a phosphoprotein able to form homotetramers. Viral RNA synthesis is dependent on functional P protein interactions with L, N and M2-1 proteins. The question raised is how P protein is tagged for establishing these interactions. To test if its phosphorylation and/or oligomerization stage is the clue, P protein variants were obtained. Binding capacity to RNPs proteins and viral transcription and replication complementation analyses of non functional P protein variants have identified residues essential for functional interactions with itself, L, N and M2-1 proteins. P protein may establish some interactions as monomer, but efficient viral transcription and replication requires P protein oligomerization. A model of a stable three-dimensional structure for the region between residues 98-158 is available. Molecular dynamics analysis of the effect of selected substitutions on the stability of the modelled tetramer confirmed by experimental analysis indicated that P protein residues L135, D139, E140 and L142 are essential for homotetramerization. Thus, compounds targeted to those residues, located in a modeled tridimensional structure, could have specific antiviral activity.

 # 102 Section: Viral Structure, Entry, Replication and Cell Biology
Title: Sequence variation within 60-nucleotide duplication: an Indian perspective
Authors: Preeti Bharaj[1], Wayne Sullender[2], Shama Praveen[1], Shobha Broor[1]

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Group B human respiratory syncytial viruses (hRSV) with 60 nucleotide duplication in G protein were first identified from Buenos Aires (BA), Argentina in 1999. These were classified as BA viruses and later identified from different countries. In earlier samples, the duplicated regions were identical to each other. However, in later isolated BA viruses, the duplicated region started to accumulate substitutions (synonymous and non synonymous). We have earlier reported these viruses from New Delhi, India. In the present study our aim was to analyze amino acid changes accumulating in the duplicated region of G protein of BA viruses after 2005 in our laboratory. During 2005-2007, there was a prevalence of hRSV group B viruses as compared to hRSV group A during 2002-2005.The 2005-2007 samples were exclusively from an urban referral hospital in New Delhi, India. We analyzed 28 hRSV group B samples (11 were already published earlier). After analyzing sequences obtained, we found twelve amino acid changes in the sequences determined during 2005-2007 as compared to four during 2002-2005. two amino acid changes S247P and T270I/V were seen in all the BA isolates. Moreover, V271A was seen in majority of our BA isolates. The length of G protein in BA viruses isolated during 2002-2005 had 312 and 319 amino acids, whereas during later samples of 2005-2007 we isolated BA viruses with 315 amino acid length typically similar to the first identified BA viruses during 1999. On average, the non synonymous mutation/synonymous mutation (*dN*/*dS*) ratio for these sequences was 1.19 as compared to 1.16 seen earlier during 2002-2005 BA isolates. Two N-glycosylation sites that have already been identified among the group B strains at the C-terminal end of the G protein gene were conserved among all the isolates. The predicted O-linked glycosylation sites in the second hypervariable region of the G protein gene analyzed in this study were 8 to 10 residues for group B viruses. These changes support the idea of a positive selection for some of the changes. Duplication tag of group B hRSV BA isolates offers an unique opportunity to study the evolution and immune pressures on this virus during propagation in its natural host.

 # 103 Section: Viral Structure, Entry, Replication and Cell Biology

Title: Respiratory Syncytial Virus Infects and Abortively Replicates in the Lungs In Spite of Pre-existing Immunity

Authors: Marina S. Boukhvalova, Gregory A. Prince, Jorge C.G. Blanco.

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Abstract: Respiratory syncytial virus (RSV) is a major cause of bronchiolitis and viral pneumonia in young children, and a serious health risk in immunocompromised individuals and in the elderly. Immunity to RSV is not completely understood. In this work we have established a method for monitoring RSV infection by real-time PCR and applied this method for analysis of RSV replication *in vivo* in the cotton rat model in naïve animals and in animals rendered immune to RSV by prior RSV infection. We found that even though no virus could be isolated from the lungs of RSV-challenged immune animals, RSV infection in fact took place and accumulation of viral RNA transcripts was observed. This type of replication, therefore, can be termed “abortive”, as RSV is capable of entering the cells in the lungs of immune animals, yet production of progeny viruses is impaired. Similar patterns of RSV gene expression gradient were observed between naïve and re-infected animals, indicating that skewing of mRNA gradient of viral gene expression, a mechanism documented during latent infection by other viruses, is not likely to be responsible for abortive replication of RSV during re-infection. We found that passive administration of antibodies to RSV prevents productive infection normally accompanied by viral release in the lung, but does not prevent abortive replication of the virus. To the best of our knowledge, this is the first evidence of abortive replication of RSV *in vivo*.

104 Section Viral Structure

RSV Regulates p53 to Delay Apoptosis in Airway Epithelial Cells

D. Groskreutz, M. Monick, T. Yarovinsky, D. Quelle, D. Look, and G. Hunninghake. The University of Iowa Carver College of Medicine and the VA, Iowa City, IA

RATIONALE: RSV infects airway epithelial cells. p53 is a tumor suppressor protein that causes apoptosis. MDM2 ubiquitinates p53 and targets it for proteasome degradation. We hypothesize that RSV decreases p53 by enhancing MDM2-mediated p53 degradation, delaying apoptosis and allowing more viral replication.
METHODS: Primary airway epithelial cells were exposed to RSV for zero to three days. p53, MDM2, phospho-MDM2, and RSV protein were detected by Western blot. p53 mRNA was quantified with real-time PCR. Endogenous p53 protein was increased using Nutlin-3, a chemical inhibitor of MDM2. Propidium iodide staining with flow cytometry quantified cell death in cells exposed to RSV and/or Nutlin-3.
RESULTS: p53 protein was decreased after 16 hours of RSV infection as shown by Western blot, but there was no change in p53 mRNA. Total MDM2 was unchanged, while the amount of phospho-MDM2 increased after 1-6 hours of RSV infection, temporally preceding the decrease in p53 protein. Inhibition of the MDM2/p53 interaction with Nutlin-3 increased p53 in control cells and cells exposed to RSV for 24 hours. This increased p53 led to an earlier and increased magnitude of cell death in RSV infected cells as measured by propidium iodide staining as well as less viral replication seen in Western blot for RSV protein.
CONCLUSION: RSV delays cell death by decreasing p53 protein via MDM2-mediated degradation. Preventing the MDM2/p53 interaction leads to increased p53 protein; earlier, enhanced cell death; and less viral replication. These observations suggest that MDM2 and p53 might be important targets for therapy in RSV infection.
VA Merit Review grant; NIH: HL073967-01, HL077431-01, R01 HL079901-01A1 and RR00059 from General Clinical Research Centers Program, NCRR, NIH.

 # 105 Section: Viral Structure, Entry, Replication and Cell Biology

Title: Vero-Grown Respiratory Syncytial Virus Contains Less Mature G Protein and Is Less Dependent on Glycosaminoglycans for Entry

Authors: Steve A. Kwilas[1,3], Liqun Zhang[4], Raymond J. Pickles[4], and Mark E. Peeples[2,3]

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Abstract: RSV produced in HEp-2 cells uses cell surface glycosaminoglycans (GAGs) for efficient infection of immortalized cells. Its G protein is the main protein involved in binding GAGs. We have found that RSV produced in Vero cells were 10-fold less dependent on GAGs for entry. Virions produced in HEp-2 cells contain primarily the 90kDa G protein. Strikingly, virions from Vero cells contain primarily a 55kDa G protein with minor amounts of 90kDa. The 55kDa precursor to 90kDa lacks O-linked sugar chains and is Endo H sensitive. However, the 55kDa G protein on the virions from Vero cells is resistant to Endo H and to O-glycanase indicating that it has passed through the Golgi without obtaining O-linked sugars. Virus produced in primary, well-differentiated human airway epithelial (HAE) cultures was GAG-dependent, like virus from HEp-2 cells, and only 90kDa was observed in HAE cell lysates. HAE cultures do not express heparan sulfate, the most important GAG for RSV binding, on their apical surface, so loss of the ability of Vero-grown RSV to bind GAGs should not be a problem. However, Vero-grown RSV infected HAE extremely poorly, indicating that 90kDa is critical for entry into respiratory cells but not because of its ability to bind GAGs. Taken together these experiments indicate that the host cell used to grow RSV may dramatically affect the infection phenotype of the RSV produced.

106 Section: Viral structure, entry, replication, and cell biology

Title: RSV infection induces a host stress granule response

Authors: Michael Lindquist[1], Philip Santangelo[2], and James E. Crowe, Jr. [1, 3]

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Abstract: RSV infection of epithelial cells is known to cause the formation of intracytoplasmic inclusion bodies, but the biology of these inclusions is not well understood. Using immunofluorescence confocal microscopy of RSV-infected or N/P-transfected cells, we determined that major proteins involved with the replication complex (N, P, M2-1) accumulate in inclusion bodies, but viral RNA concentrates in separate granules. Our data show that RSV infection of HEp-2 cells induces the accumulation of host RNA processing proteins into cytoplasmic stress granules containing RSV RNA that often are juxtaposed with RSV inclusion bodies. Our experiments also reveal that multiple host processing body proteins are also relocalized into RSV-induced stress granules. Remarkably, expression of the RSV nucleoprotein and phosphoprotein by transfection was sufficient to induce stress granule formation and to mediate interaction of viral inclusion bodies with host stress granules. These data reveal novel features of the epithelial cell host response to RSV infection, and provide insight in to mechanisms of replication.

#107 Section: Viral Structure, Entry, Replication and Cell Biology

Title: Reduction of HRSV budding and protein production in multiple cell lines by treatment with proteasome inhibitor MG132

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Abstract: We have found that the release of HRSV virus from host cells requires factors that are believed to be recruited to the site of budding by the Matrix (M) protein. Using yeast two-hybrid, we have shown that the M protein of HRSV interacts with host ubiquitin encoded by the polyubiquitin gene UbC. HRSV infected Vero and HEp-2 cell cultures treated with the proteasome inhibitor MG-132 at 4, 14, or 24hr post infection show a time and dose sensitive decrease in virus budding and protein production. Budding was reduced by 50-75% depending on the cell line. Likewise, viral protein production was reduced by 50-70%. The combinatorial effects of decreased budding and protein production were confirmed by a 3.42 and 1.99 log reduction in plaque titers. The budding of other viruses, such as SV5 and HIV, have previously been shown to be sensitive to MG132. Among those viruses tested, however, HRSV is currently the only virus to display a reduction in protein production due to treatment with a proteasome inhibitor. This reduction appears to be specific for viral proteins, as there was no correlation between treatment and β-Actin or GAPDH loading control protein levels. In addition, the reduction in viral proteins is not likely due to a Type I Interferon mediated response, as Vero cells also displayed a specific reduction in viral proteins. The mechanism for this reduction in viral replication is unknown at this time. These results indicate that ubiquitin is indispensable for proper replication and budding of HRSV in multiple cell lines and may play a major role in HRSV pathogenesis.

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