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RSV 2007: Recent Advances in Respiratory Syncytial Virus Research

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ABSTRACT

THE SIXTH INTERNATIONAL RESPIRATORY SYNCYTIAL VIRUS (RSV) SYMPOSIUM was held from October 25 through October 28, 2007 in Marco Island, Florida. During this conference, over 200 students and investigators representing 16 countries convened to present and discuss recent advancements in RSV research. Presentations ranged from bench to bedside studies encompassing aspects of basic virology, pathogenesis, and immunology, as well as therapeutic and vaccine designs. In total, there were 12 invited speakers and 36 oral presentations divided into six sessions. Additionally, there were two posters sessions displaying more than one hundred abstracts. The following summary provides a review of the proceedings from each session.

VIRAL STRUCTURE, ENTRY, REPLICATION, AND CELL BIOLOGY

Dr. Gail Wertz from the University of Virginia (Charlottesville, VA) began the meeting by delivering the Robert M. Chanock Lecture entitled "Natural and Experimental Evolution of Human RSV." In her lecture on the history of RSV research in her laboratory, she recounted not only the work, but also the colleagues and collaborators who were involved in the work that led to the first molecular description of the RSV genome and its encoded gene products and numerous experiments that led to a greater understanding of RSV replication and its role as a pathogen and modulator of the host immune response. Some of her laboratory's contributions include cloning the genes of RSV, determining the physical and transcriptional map of the RSV genome, elucidating the transcriptional regulation of RSV, identifying novel factors involved in RSV antigenic variation, and engineering vaccinia virus vectors that contain the coding sequence of individual RSV proteins. As an interesting aside, she mentioned that approximately 400 requests to date have been made for such vaccinia virus constructs. Taken together, her seminar beautifully exemplified the impact of her work on the field of RSV research through both direct and indirect contributions.

Some of the current investigations within the Wertz laboratory include engineering recombinant RSV deletion mutants that lack either one or all of the viral glycoproteins. To this end, Melissa Batonick (University of Virginia, Charlottesville, VA) presented data utilizing this virus to address the role RSV glycoproteins may play in the apical targeting and release of the RSV virion in polarized epithelium. Her studies indicate that the RSV fusion (F), attachment (G), and small hydrophobic (SH) proteins together and individually are not directly responsible for regulating the apical sorting or release of virions.

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There were two presentations addressing biochemical and structural mechanisms involved in viral fusion. José Melero (Instituto de Salud Carlos III, Madrid, Spain) first presented structural data identifying two cleavage sites on the RSV F protein that are required for fusion with cell membranes. He also examined possible mechanisms explaining the unique capability of RSV to undergo fusion without the use of its attachment protein. By modeling the F protein during various stages of viral fusion, he discovered, during an intermediate stage, a newly exposed site on the F protein that is distinct from other defined structural areas. Additionally, this site was shown to be capable of viral fusogenic activities and current studies in his laboratory are further investigating the potential implications of this site. Next, Theodore Jardetzky (Stanford University, Palo Alto, CA) examined how the structure of F correlates with its function during the initial stages of viral entry. He observed that the pre- and post-entry forms of the F protein display remarkable differences in their conformations, especially in secondary and tertiary structure. These findings suggest how the F protein mediates the fusion of the viral and lipid bilayers during virus entry and highlight specific features of the RSV F protein that may confer its unique functional properties within the Paramyxovirus family.

The final talk in this session described novel data examining the seasonality of RSV epidemics in long-term cultures of RSV-infected monocyte-derived dendritic cells. Mark Everard (Sheffield Children's Hospital, Sheffield, U.K.) observed that RSV replication in these cells paralleled the seasonal pattern of RSV epidemics, but could also be observed during non-RSV seasons following the administration of nitric oxide to the cultures. Taken together, he suggests that dendritic cells may function as reservoirs for RSV outside of the RSV season, and that viral replication may be induced in this cell type by a drop in endogenous nitric oxide production.

IMMUNOLOGY: INNATE AND ADAPTIVE

Many studies have indicated that the RSV F protein can bind to toll-like receptor (TLR) 4 and thus activate the innate component of the host immune response. However, the type of immune response generated during RSV infection differs from that observed following stimulation with other TLR4 agonists, for example LPS. Fernando Polack (Johns Hopkins University, Baltimore, MD) examined the role that individual RSV proteins may play in modulating the host immune response. He presented data identifying a cysteine-rich region of the RSV G protein that dampens the adaptive immune response generated against the RSV F protein. Additionally, he found that the F protein, through engagement of TLR4, is able to promote the affinity maturation of RSV neutralizing antibodies. Taken together, he identified multiple mechanisms by which certain RSV proteins could reduce the degree of inflammation induced following the ligation of TLR4. At the close of his presentation, he also included population-based data studying RSV disease in high-risk populations and different socioeconomic classes in Argentina. These findings suggest an inverse correlation between disease severity, TLR4 functionality, and socioeconomic status. Taken together, it is hypothesized that genetic locus variation (i.e., TLR4 mutations) and the environment are both critical determinants in identifying which populations may be at a higher propensity to develop severe RSV disease.

Riny Janssen (National Institute for Public Health, Bilthoven, The Netherlands) shared information from a genetic study aimed to further elucidate which host genes and biological pathways are involved in determining susceptibility to severe RSV bronchiolitis. Following examination of 347 single nucleotide polymorphisms (SNPs) in over 200 candidate genes from children hospitalized for RSV bronchiolitis, she found a strong association between SNPs in genes involved in the innate immune response (i.e., VDR, JUN, IFNA5, and NOS2) and development of RSV bronchiolitis. Jennifer Reed (MedImmune, Gaithersburg, MD) proposed that alveolar macrophages could also be a determinant regulating the degree of RSV disease severity. She performed RSV infection in mice with constitutive macrophage impairment, and in wild-type mice in which alveolar macrophages were depleted with clodronate. Her data show that in the absence of functional pulmonary macrophages, there are increases in RSV titers and type I interferon–associated cytokine production by pulmonary epithelium. Airways occlusion with cell debris and increased inflammatory cell recruitment are also observed. She hypothesizes that alveolar macrophages regulate RSV disease severity by limiting the infection of pulmonary epithelium and by engulfing apoptotic cells. These activities curtail subsequent inflammatory cell infiltration and development of bronchiolar occlusions.

In some models of RSV infection, a robust T-cell response is generated that has critical yet paradoxical roles in mediating both viral clearance and RSV disease pathogenesis. In order to better understand the role $CD8^+$ T cells may play in these processes, Grada Van Bleek (Utrecht University, Utrecht, The Netherlands) presented data examining the tempo and quality of the RSV-specific $CD8^+$ T-cell response in infants undergoing severe primary RSV infections. Her work demonstrates a robust systemic $CD8^+$ T-cell response observed peaking in the peripheral blood surprisingly, at the time of RSV resolution. Prior to this time point, it is hypothesized that memory effector T cells traffic from the blood into the lungs to mediate antiviral effects.

Separate work has previously shown that the responding RSV-specific $CD8^+$ T cells in the lungs during a primary or secondary RSV challenge in mice are defective as measured by a decrease in IFN-γ production. Jonathan Castillo (University of Virginia, Charlottesville, VA) used genetically engineered recombinant RSV mutants lacking specific RSV proteins described previously to determine the role certain RSV proteins may have in modulating the $CD8^+$ T-cell response. His study demonstrates that the RSV F, SH, and G proteins are not involved in the suppression of $CD8^+$ T-cell effector responses and current studies are underway further exploring the mechanism involved.

Peter Openshaw (Imperial College London, U.K.) presented data addressing how T cells generated during a neonatal RSV infection can impact the nature and quality of disease observed upon RSV re-infection in adulthood. During primary RSV infection of neonatal mice, $CD8^+$ T cells were shown to lead, by an unknown mechanism, to the generation of poor booster antibody responses and an augmented disease phenotype during secondary RSV challenge. In contrast, $CD4^+$ T cells had no effect on antibody generation in the neonatal period but were required for boosting to occur during secondary RSV infection.

RSV disease affects not only human populations, but also has a profound impact on cattle and the agriculture industry as a whole. Geraldine Taylor (Institute for Animal Health, Compton, U.K.) examined RSV infections in cattle and discussed her findings discovering the first bovine RSV (bRSV) epitope recognized by bovine $CD8^+$ T cells. This epitope is a BoLA N*01301 (A18)-restricted 9-mer peptide corresponding to amino acids 141–149 of the bRSV P protein. Interestingly, she also presented data illustrating an increase in bRSV-specific $CD4^+CD8^+$ T cells following serial re-stimulations with bRSV-infected macrophages, *in vitro*. Since studies in humans and mice have suggested that these double positive cells enhance effector functions, current studies are underway to examine the role of bRSV-specific $CD4^+CD8^+$ T cells in cattle during bRSV infections.

To conclude this session, Michelle Gill (University of Texas Southwestern, Dallas, TX) presented work examining MHC class II expression on the surface of human antigen-presenting cells isolated from nasopharyngeal samples from infants hospitalized with RSV bronchiolitis. She reported a transient downregulation of MHC II expression on certain dendritic cell subsets and monocytes during acute RSV infection. Additionally, *in vitro* studies suggest that the downregulation of MHC II expression by dendritic cells is dependent on the dose of virus administered and is possibly mediated through the inhibition of IFN-γ signaling.

PATHOGENESIS

The first speaker of this session was Stefanie Vogel (University of Maryland, Baltimore, MD), who investigated the relationship between TLR4 signaling and RSV disease. On a cellular level, her work identified two single nucleotide polymorphisms (previously associated with decreased LPS sensitivity) that were predicted to lie on the same face of TLR4 and affect interactions with other TLR4 signaling molecules. This was based in part on their observation that three structurally distinct TLR4 agonists, including RSV F protein, induced diminished activation of NF-κB in a transfection system. Vogel also presented population-based studies examining the predominance of such TLR4 SNPs in infants and children at high risk for RSV who were confirmed to have had symptomatic RSV. Her data indicate a surprisingly strong correlation between symptomatic RSV disease in this case series and the expression of the Asp299Gly and Thr399Ile SNPs. She hypothesizes that TLR4 deficiency may affect not only sensitivity to RSV disease, but also aspects of neonatal development leading to "high-risk" status (e.g., prematurity). Lastly, they found that administration of the non-toxic TLR4 agonist, monophosphoryl lipid A (MPL), to cotton rats with formalin-inactivated RSV, blunts vaccine-enhanced disease by blunting the ensuing cytokine storm that is comprised of both Th-1 and Th-2 cytokines.

A recently identified respiratory virus with a similar clinical presentation to RSV is human metapneumovirus (hMPV). Roberto Garofalo (University of Texas, Galveston, TX) presented data comparing various characteristics of the host immune response to either infection. For example, he shows that while human monocyte-derived dendritic cells were susceptible to infection by either hMPV or RSV, the type of cytokines produced by infected dendritic cells varied depending on viral species. Interestingly, this study also describes a potentially opposing role for alveolar macrophages following RSV or hMPV infection. It is hypothesized that, while alveolar macrophages are the major source of type I interferon cytokines in the lungs in either infection, this cell type may promote antiviral responses in RSV infection while enhancing viral replication in hMPV infection.

In this session, several mechanisms RSV may employ to evade the host immune response were also addressed. Alexander Bukreyev (National Institute of Allergy and Infectious Diseases, Bethesda, MD) examined the role of the soluble RSV G protein in virus-mediated immune evasion. He observed that the recombinant RSV with the deletion of the soluble G protein has an increased susceptibility to RSV neutralizing antibodies both *in vitro* and in mice injected with the antibodies. Thus, the soluble G protein is hypothesized to mediate evasion of RSV

from the host's immune system. Additionally, Kizzmekia Corbett (National Institute of Allergy and Infectious Diseases, Bethesda, MD) reported that the soluble RSV G protein can interact with primary human dendritic cells, altering surface expression of various co-stimulatory molecules and production of certain cytokines. She also provided evidence that RSV preferentially infects myeloid dendritic cells at a higher frequency than plasmacytoid DCs, but only in the presence of divalent cations. RSV exposure induced dendritic cell maturation and cytokine production that was not dependent on viral replication, and in a subset of donors inhibited TLR-mediated events.

Winner of the best abstract award, Christine Oshansky (University of Georgia, Athens, GA), studied how RSV may selectively regulate host gene expression through interactions with suppressor of cytokine signaling (SOCS) proteins. She showed that, following RSV infection in normal human bronchial epithelial cells, SOCS1 and SOCS3 are rapidly induced and there is a decrease in type I interferon expression. She hypothesizes that RSV F-mediated activation of the TLR4 pathway can lead to the hyperphosphorylation of the interferon regulatory factor (IRF)-3 and the upregulation of SOCS expression, which in turn regulates the amount of type I interferons produced during RSV infection.

The final speaker of this session, Albert Senft (Lovelace Respiratory Research Institute, Albuquerque, NM) presented work illustrating a dose- and time-dependent increase in the phosphorylation of STAT-1β following IFN-γ stimulation of RSV-infected macrophages. This in turn led to a decrease in the nuclear STAT-1 interaction with CBP/p300 and a reduction in the transcription of CIITA. These findings suggest that the combined effects of RSV infection and IFN-γ stimulation may impair the ability of macrophages to process and present antigen on MHC complexes, thereby enhancing RSV-mediated immune evasion.

PATHOGENESIS II AND PULMONARY ASPECTS OF RSV

Gary Hunninghake (University of Iowa, Iowa City, IA) examined the role of pulmonary epithelium in shaping the development of the host immune response to RSV infection. RSV-infected airway epithelial cells were shown to increase their expression of the epidermal growth factor receptor (EGFR), leading to a subsequent increase in epithelial cell production of proinflammatory cytokines such as IL-8 and pro-survival proteins such as Bcl-xl. Interestingly, under Th-2 polarizing conditions, RSV has also been shown to induce pulmonary epithelial cell production of the chemokine TARC, which leads to the further recruitment of Th-2 polarized cells into the lungs and completes a positive feedback loop between RSV infection and Th-2 cytokines. In summary, these data suggest that RSV can influence the subsequent development of an immune response in part by altering pulmonary epithelial cell survival and proinflammatory cytokine production.

Nick Lukacs (University of Michigan, Ann Arbor, MI) presented evidence highlighting the importance of the notch ligand, delta-like 4 (dll4), on dendritic cells in regulating the development of a $CD4^+$ T-cell Th-2 type response during RSV infection. By developing a polyclonal antibody against dll4, his laboratory showed that wild-type RSV-infected mice depleted of dll4 developed increased airway hyperresponsiveness, heightened pulmonary eosinophil infiltrate, and a skewing toward Th-2 cytokine production by $CD4^+$ T cells. From this and other observations, he hypothesizes that the dll4 notch ligand on dendritic cells plays an important role in attenuating the development of a $CD4^+$ T-cell Th-2 type immune response during RSV infection, and current work is underway to investigate the mechanism by which this may occur.

Raymond Pickles (University of North Carolina, Chapel Hill, NC) discussed the importance of mucociliary clearance in the airways following RSV infection. By culturing ciliated human airway epithelial (HAE) cells, he observed that the clearance of virus and debris by ciliated beat effectiveness was impaired if the cells were defective in ion-transport (i.e., contained a mutation in the CFTR ion channel gene). From this work, it is hypothesized that RSV-infected patients with chronic lung disease such as cystic fibrosis have a diminished ability of ciliated-epithelium-mediated clearance of virus and other apoptotic debris in the airway, which leads to reduced viral clearance and further exacerbation of disease pathogenesis. In summary, these observations help to identify why patients with chronic lung disease have an increased severity of virus-induced consequences leading to exacerbation of lung disease.

Michael Chi (Vanderbilt University School of Medicine, Nashville, TN) presented data supporting the idea that unique strains of co-circulating RSV may differentially influence the development, extent, and quality of RSV pathogenesis. For example, he showed that the A/Nashville/2001/2-20 RSV strain induced a severe disease phenotype in BALB/c mice, as measured by factors such as weight loss, increased breath distension, and increased mucus production, when compared to RSV strains A/Nashville/2001/3-12, A2, Long, and line 19.

Xiaoyong Bao (University of Texas Medical Branch, Galveston, TX) examined the mechanism by which the hMPV G and SH proteins lead to an inhibition of chemokine and type I interferon production by human

epithelium using deletion mutant or recombinant wild-type viruses. These data indicate that upon infection with a mutant hMPV lacking the G protein, there is an enhanced activation of transcription factors belonging to the IRF-3, AP-1, and NF-κB families. Additionally, this study indicates that upon infection with a mutant hMPV lacking the SH protein, there is a heightened activation of NF-κB family–dependent genes. These findings suggest that both the hMPV G and SH proteins may serve as regulators of early cytokine signaling that lessen the production of proinflammatory cytokines and assist in viral immune evasion.

One highly debated aspect of RSV disease pathogenesis is whether RSV re-infection is associated with post-bronchiolitis wheezing. To address this issue, A. Schuurhof (University Medical Center Utrecht, Utrecht, The Netherlands) presented data from a 6-month clinical study monitoring respiratory tract symptoms and wheezing episodes in infants who were previously hospitalized with RSV bronchiolitis. The data from this study showed that wheezing episodes are always associated with the presence of at least one respiratory virus in the airways. However, there is no direct association between RSV re-infection and the development of post-bronchiolitis wheeze.

At-risk populations for severe RSV infections include not only premature infants, but also elderly patients with confounding morbidities. Marina Boukhvalova (Virion Systems, Inc., Rockville, MD) presented work analyzing how age may influence the development of RSV pathology. Even though both antiviral and anti-inflammatory treatments were shown to have similar effects on cytokine production in both age groups, these data indicate that the peak in cytokine production observed in aged RSV-infected cotton rats occurs later during the course of infection than in RSV-infected younger controls. Additionally, this study shows that in older animals, there is an increase in GRO chemokine expression in the lungs when compared to younger animals. Future work will continue to study how the age-related discrepancies in cytokine production delay and certain chemokine expression levels may regulate RSV disease pathogenesis.

Arno Andeweg (Erasmus Medical College, Rotterdam, The Netherlands) showed results from a microarray analysis examining the expression patterns of host innate response genes from cultured lung epithelial cells infected with various respiratory viruses. These data revealed that RSV, hMPV, and influenza each induce a strong innate response. However, only influenza infection was shown to downregulate host mRNA levels. In summary, these data will provide a foundation for future studies examining in greater detail the interactions between certain respiratory viruses and their hosts.

To conclude this session, Whitney Stevens (University of Virginia, Charlottesville, VA) presented work examining the role memory $CD8^+$ T cells may play in influencing the development of RSV vaccine-enhanced disease. By studying the tempo of the RSV-specific $CD8^+$ T-cell response to secondary RSV infection in mice, her work suggests that the early recruitment of RSV-specific $CD8^+$ T cells into the lungs from the draining lymph nodes may be sufficient in modulating the $mCD4^+$ T-cell Th-2 type response attenuating the development of pulmonary eosinophilia and thus RSV vaccine-enhanced disease.

CLINICAL ASPECTS OF RSV AND VACCINES

Octavio Ramilo (University of Texas Southwestern Medical Center, Dallas, TX) discussed how respiratory viruses have unique "fingerprints" that can be identified by measuring variances in host gene transcription levels following infection. For example, by using a gene array analysis of cells harvested from the blood of infected patients during the acute phase of infection, this study shows that influenza infection leads to the enhanced expression of interferon-related genes when compared to levels observed during RSV infection, which showed increased expression of neutrophil-related genes. Additionally, this technique was also shown capable of discriminating between mild and severe lower respiratory tract RSV infections. In summary, by using gene arrays to identify unique transcriptional profiles in patients with respiratory tract infections, the infectious agent can be more rapidly and easily identified. This, in turn, will aid in determining the appropriate therapeutic course of action.

Janet Englund (University of Washington, Seattle, WA) examined the incidence and rate of spread of RSV in a day-care setting as well as the impact RSV disease has on the infected infant and their family. This study indicates that, while RSV is not the most predominant virus isolated during the winter months in a specific day care, RSV does have the greatest clinical impact on the degree of clinical symptoms observed (i.e., fever and wheezing), the number of medical visits prompted, the number of days the infected infant is absent from day care, and the number of days the infected infant's parents are absent from work. Additionally, following the documentation of the first episode of RSV, this study showed how quickly RSV can spread from room to room at the day care.

While there are some available therapeutics for the prevention and treatment of severe RSV disease, many of these are cost prohibitive, especially in communities of low socioeconomic status. As a result, Fernando Polack (Johns Hopkins University, Baltimore, MD) presented

data studying how breast-feeding may impact a high-risk infant's susceptibility to developing severe acute lung disease following RSV infection. This study found that breast milk could protect females, but not males, from severe respiratory infections. Furthermore, this study suggests that premature non-breast-fed females are the most at-risk group for developing severe acute lung disease following RSV infection. Studies are currently underway to further examine how breast milk is able to confer protective gender-specific immunity.

Neutrophils represent the predominant cell population responding during RSV infection. C.P. Halfhide working with R.L. Smyth (University of Liverpool, Liverpool, U.K.) investigated the presence of RSV proteins on neutrophils in infants hospitalized with severe RSV bronchiolitis. They showed that in infants with severe RSV infection, RSV proteins were found on neutrophils in the BAL fluid and blood, suggesting that RSV can bind to neutrophils during active RSV disease.

Edward Walsh (University of Rochester, Rochester, NY) studied the prevalence of certain respiratory infections in a community of non-hospitalized adults of varying ages and morbidities, and in patients hospitalized for acute respiratory symptoms. The results of this study suggest that hMPV re-infections are common throughout adulthood, and in young adults, mostly have asymptomatic presentations. Among hospitalized patients, influenza, RSV, and hMPV rank as the first, second, and third most common agents identified, respectively. This study indicates that hMPV is an important viral pathogen among adults, especially in the elderly and hospitalized populations.

Unfortunately, there are no licensed RSV or hMPV vaccines currently available. Additionally, there are many hurdles facing the development of such vaccines, including the young age at which vaccination must occur, and for RSV, the past history of a failed vaccination strategy that led to severe immunopathology upon natural re-infection. This meeting stressed the necessity for further vaccine research, as well as emphasized several encouraging advances in the field of RSV and hMPV vaccine design.

First, a replication-defective adenovirus vector-based vaccine strategy expressing selected RSV proteins was described by Barney Graham (National Institutes of Health, Bethesda, MD). Vaccination by gene delivery using a vector-based approach provides advantages for avoiding maternal immunity and aberrant immune responses through the control of antigen content, vector selection, and delivery. One such vector, rAd5, was shown to mature primary human dendritic cells and generate an effective innate response *in vitro*. In a murine model, Ad5 vectors expressing M/M2, a model T-cell antigen, induced strong $CD8^+$ CTL responses and a Th-1 $CD4^+$ T response pattern that effectively decreased viral replication and illness. These data support further evaluation of replication-defective adenovirus vectors and other gene-based vectors, including consideration of future vaccine clinical trials.

Next, Peter Wright (Vanderbilt University, Nashville, TN) presented data from a clinical trial studying the response to natural infection after receipt of several live attenuated RSV vaccines that had been administered to previously uninfected children ranging from either 6–24 or 1–3 months of age. An "intent to treat" analysis demonstrated a decrease in the rate of RSV-associated upper and lower respiratory tract infections in vaccinated compared to the control populations in each age group. Importantly, there was no evidence that these vaccines led to enhanced disease upon re-infection. This work has provided basic evidence that live attenuated RSV vaccines can be safely used in future vaccine clinical trials.

In the final talk of this session, Sander Herfst (Erasmus Medical Center, Rotterdam, The Netherlands) presented data examining two hMPV vaccine candidates. A cold-adapted temperature-sensitive live attenuated hMPV vaccine was shown to induce high titers of hMPV-specific antibodies upon administration. Following challenge infection, vaccinated animals were protected as evident by a decrease in viral replication in the upper respiratory tract and no viral replication detected in the lower respiratory tract. The second vaccine, an hMPV F subunit vaccine, was also found to confer resistance after challenge infection of vaccinated animals as demonstrated by high neutralizing antibody titers, significantly reduced hMPV viral titers in the upper respiratory tract, and complete protection of the lower respiratory tract. Taken together, these data suggest that immunization with either the live attenuated or subunit vaccine can induce protection against lower respiratory hMPV infections in animals.

THERAPEUTICS AND VACCINES

Continuing the discussion of vaccines and therapeutics, John DeVincenzo (University of Tennessee, Memphis, TN) opened this session by describing ALN-RSV01, an siRNA compound targeted toward the RSV N, P, and L proteins required for viral replication. This compound, given either before or following RSV infection in animal trials, was observed to reduce viral replication in the lungs of treated animals. Additionally, ALN-RSV01 was also found to be protective when different strains of RSV were administered. In a single-dose randomized double blind clinical trial, the first known trial in which siRNA targeting a virus has been tested in humans, ALN-RSV01 was safely tolerated. Current studies are underway to further study the efficacy of this compound.

As a means to test the effectiveness of potential RSV vaccines and antiviral therapeutics, DeVincenzo described a "standardized" experimental human RSV strain developed in his laboratory. This virus was shown to have similar characteristics to the naturally occurring RSV strain, and also provided consistently reproducible viral endpoints and disease symptoms. Importantly, this experimental strain did not generate any adverse effects. In summary, this experimental RSV strain could provide a universal and reliable standard to measure clinical trial efficacy.

Wenliang Zhang (University of Georgia, Athens, GA) examined how small interfering RNA prophylaxis affects the primary and memory immune responses to RSV infection. This study indicated that siRNA treatment targeting the RSV P gene reduces viral titers, disease pathogenesis, and total cellular infiltrate detected in the BAL fluid following RSV infection. Additionally, this therapy induced an enhanced memory $CD4^+$ and $CD8^+$ T-cell response upon RSV re-infection, characterized by heightened T-cell kinetics as well as an increase in their cytokine production. In summary, this study suggests that siRNA prophylaxis can enhance the generation of a potent memory response as well as mediate antiviral effects.

Leon de Waal (Erasmus Medical College, Rotterdam, The Netherlands) compared the efficiency of more traditional read-outs of disease severity with those obtained using a microarray analysis comparing mRNA expression profiles in RSV-infected BALB/c mice primed with either live RSV or with vaccinia virus constructs expressing the RSV F, G, or M2 proteins. His data indicate that microarray analysis can identify phenotypic-specific expression profiles consistent with those observed in the more traditional immunological or virological based assays. Also, by comparing mRNA expression profiles, certain individual genes were identified that may be important in the development of specific responses. In summary, these data provide a foundation for how future studies could be performed to analyze the host immune response to various candidate vaccines in high resolution.

Ann Falsey (University of Rochester, Rochester, NY) described a phase II clinical trial addressing (1) the immunogenicity of an RSV subunit vaccine containing the F, G, and M proteins with or without adjuvant in at-risk elderly populations, and (2) any potential interference that may arise when given concomitantly with a licensed influenza vaccine. This study observed that the RSV vaccine was well tolerated in patients and was moderately immunogenic. The non-adjuvanted formulation was superior to the adjuvant formulation as determined by increased post-vaccination neutralizing antibody titers. However, antibody titers in both vaccination groups returned to baseline by 1 year, necessitating yearly boosting. Importantly, this study showed that either RSV vaccine formulation did not interfere with the influenza humoral response.

In addition to having no licensed RSV or hMPV vaccines, there are also no widely utilized antiviral therapies against these viruses available as treatment options. Daniel Pevear (Novartis, Cambridge, MA) opened the discussion on therapeutics with a very informative presentation focusing first on the stages of drug development beginning with conception and continuing through FDA approval. Additionally, Pevear presented an overview of several anti-RSV compounds currently in various stages of pre-clinical and clinical development, including four inhibitors of viral entry and three inhibitors of viral replication.

Another potential RSV therapeutic discussed was an anti-inflammatory, experimental immunosuppressive agent, leflunomide. Melinda Dunn (The Ohio State University, Columbus, OH) presented data showing that the administration of this drug, either concurrently with RSV inoculation or 3 days post-infection, reduced viral load by 3–4 logs in the lungs of treated animals by an as yet undefined mechanism. Next, Wieslawa Olszewska (Imperial College London, U.K.) tested the *in vivo* efficacy of an RSV F protein inhibitor, TMC353121 (Tibotec, Mechelen, Belgium), given either prior to or after RSV infection in BALB/c mice. Both prophylactic and therapeutic administration of the drug was shown to reduce weight loss, viral replication, and inflammatory infiltrate in the BAL fluid. Overall, this compound had antiviral effects similar to those of palivizumab, a currently licensed monoclonal antibody specific for the fusion protein (F protein).

There were also several promising studies at this meeting discussing the development of new antiviral antibodies against RSV. For example, Johan Lantto (Symphogen A/S, Lyngby, Denmark) presented work involving recombinant fully human polyclonal antibodies against RSV collectively termed Sym003. This antibody cocktail was shown to have potent viral neutralizing activities, recognize a broad array of epitopes on both the RSV F and G proteins, and to potently reduce RSV replication *in vivo*. Sym003 also has a potential for blocking the immunomodulatory effects of the F and G proteins and reduce RSV-mediated pathogenesis.

Next, Subramaniam Krishnan (MedImmune, Gaithersburg, MD) described motavizumab, a humanized IgG_1 monoclonal antibody derived through affinity maturation from palivizumab. In *in vitro* assays, it was shown that motavizumab has the potential to bind to monocytes thus influencing their production of various chemotactic agents, as well as promoting their clearance of virus-infected epithelium.

Interestingly, Geoffrey Toms (Newcastle University, Newcastle upon Tyne, U.K.) examined the relationship

between viral passages and neutralization ability by anti-F antibodies. He showed that viral adaptation to cell culture selects for neutralization-susceptible quasi-species variants, with clones from earlier passages being more resistant to neutralization by the anti-F antibody palivizumab than clones isolated from later cell passages. However, upon sequence analysis, there were no consistent changes in the F gene or F-G intra-gene sequences between resistant and susceptible clones. In summary, these data suggest there is a RSV-specific product, which is lost upon adaptation to cell culture, that confers resistance to anti-F antibodies and may have broad implications in the future designs of therapeutics.

FUTURE DIRECTIONS

The Sixth International RSV Symposium showcased novel, exciting, and promising advances in basic and clinical RSV and hMPV research. More importantly, the meeting setting catalyzed discussions and interactions among investigators interested in both the basic and clinical aspects of RSV infection and disease pathogenesis. There is no doubt that the positively charged atmosphere sparked the development of original ideas and even completely new avenues of investigation. We eagerly anticipate learning about such discoveries at the Seventh International RSV Symposium to be held in 2010 in the Netherlands.

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