

INFECTIONS AND LUNG DISEASES

▷ EPITHELIAL INFLAMMATORY RESPONSES TO RESPIRATORY BACTERIAL PATHOGENS

Human mucosal surfaces are often colonized with a wide array of microorganisms simultaneously. The epithelial cells that line these spaces act as a physical barrier and also play a vital role in the initiation of local innate immune responses. Polymicrobial stimulation of airway epithelial cells *in vitro* or *in vivo* with two mucosal pathogens, *S. pneumoniae* and *H. influenzae*, leads to synergistic induction of proinflammatory signaling, and the resultant local recruitment of neutrophils influences the outcome of interbacterial competition. Epithelial sensing of combinations of bacteria, as are commonly encountered during colonization, can drastically alter local inflammatory responses and determine the outcome of competition between bacterial species. Interventions such as vaccination or antibiotic therapy that target specific members of the respiratory flora may inadvertently alter the dynamics of complex microbial communities.

Adam J. Ratner, *University of Pennsylvania, Philadelphia, USA*

▷ IMMUNE DEFENSES IN THE RESPIRATORY EPITHELIUM

The airway mucosa is mainly covered by a thin specialized epithelium, constituting a vulnerable barrier, continuously being bombarded by exogenous antigens including potentially infectious agents. Adequate mucosal protection depends on intimate co-operation between adaptive immunity and innate defense mechanisms such as mucus and the ciliary function. Adaptive surface defense is primarily mediated by secretory IgA but also by potentially proinflammatory serum-derived and locally produced IgG antibodies. Mucosally induced regulatory mechanisms aim at avoidance of excessive inflammation and the commensal microbiota contributes to homeostatic control. There is currently considerable interest in exploiting the mucosal route both for anti-infective vaccines and immunotherapy and there is a need for more basic knowledge to this end.

Per Brandtzaeg, *University of Oslo, Norway*

▷ MOLECULAR MECHANISMS OF EPITHELIAL SIGNALING IN RESPONSE TO *S. AUREUS* AND *P. AERUGINOSA*

Airway epithelial cells are an important component of the mucosal immune system initiating the innate immune response to inhaled pathogens. To cope with the diverse types of bacterial pathogens specific receptor complexes are actively mobilized to the apical surfaces of airway cells and presented in conjunction with signaling kinases, within the context of lipid rafts. AsialoGM1 and TLR2 are co-receptors capable of recognizing and initiating NF- κ B dependent gene transcription in response to both Gram-negative and Gram-positive organisms. TNFR1 is mobilized in response to staphylococcal protein A initiating TNF signaling in response to a discrete ligand. Multiple signaling cascades may be initiated by bacterial ligands in sufficient quantity to activate these receptors.

Alice Prince, *Columbia University, New York, USA*

▷ PULMONARY INFECTIONS AND TOLL-LIKE RECEPTORS

The Toll-like receptors (TLR) are important for the innate immune response of the host to infections. Their main function is to sense pathogens and to trigger a cascade of events leading to the expression of inflammatory molecules useful for an early acute defense but harmful when produced in excess. As lungs are constantly exposed to airborne pathogens, the innate host defense is of particular importance. TLR are expressed at the lung level by different cell types, including alveolar macrophages and respiratory epithelial cells. Their role for the fight of bacteria, fungi and viruses is evident but not univocal.

Michel Chignard, *Institut Pasteur, Paris, France*

▷ TOLL-LIKE RECEPTOR SENSING AND CONTROL OF INTRACELLULAR PATHOGENS

Microbes and their products are sensed by cells of the innate immune system through Toll-Like receptors (TLR). Pathogen induced activation of antigen presenting cells recruits lymphocytes to mount an adaptive immune response with effective elimination of the pathogen. The role TLR mediated sensing and activation has been investigated for G⁺ and G⁻ bacteria, fungi, parasites and viruses. Our team focussed on *Mycobacterium tuberculosis* (MTB) infection using mice deficient for the common TLR adaptor protein, MyD88, involved in TLR signalling. MyD88^{-/-} macrophage do not respond to mycobacteria, display reduced phagocytosis and defective killing of bacilli. Aerosol infection of MyD88^{-/-} mice with MTB (100CFU) is lethal within 4 weeks with 3 log higher CFU in the lung and acute, necrotic pneumonia despite the induction of an adaptive immunity. Similar data are obtained for another intracellular pathogen, *Listeria monocytogenes*. These data suggest that TLR/MyD88 signalling plays a critical role for the activation of the innate immune system by MTB or *Listeria*, while the generation of an adaptive immune response is TLR independent.

Bernhard Ryffel, *Institut Transgenose, Orléans, France*

▷ NOVEL HETERODIMERIC CYTOKINES: MASTER REGULATORS OF LUNG IMMUNITY

Successful host defenses against invading pathogens require coordinated interactions between innate and adaptive immunity. IL-12 and IL-23 are myeloid derived heterodimeric cytokines which regulate specific aspects of innate and adaptive immunity in the lung. IL-12 is required for regulation of Th1 responses and host defense against intracellular pathogens, whereas IL-23 is required for Th17 responses and host defense against extracellular Gram negative bacteria in the lung. The regulation of IL-12 and IL-23 as well as downstream mediators of host defense, in the context of pulmonary infection, will be presented.

Jay K. Kolls, *Children's Hospital of Pittsburgh, USA*

▷ LIPID ANTI-INFLAMMATORY MEDIATORS AND THE CF LUNG

Dysregulated neutrophilic inflammation and chronic infection lead to progressive destruction of the airways in cystic fibrosis. The lipoxins are endogenous anti-inflammatory lipid mediators that are important for immune counter-regulation in the lung. Recent data indicate that there is a pathophysiologically important defect in lipoxin-mediated anti-inflammatory activity in the cystic fibrosis airway, suggesting novel approaches to pathogenesis and therapy in this lethal genetic disease.

Christopher Karp, *University of Cincinnati, USA*

▷ COMPLEMENT ANAPHYLATOXINS AND RECEPTORS IN LUNG HOST DEFENSE

The C3a and C5a molecules play complex roles in host defense. In some instances, C5a is requisite for acute clearance of bacteria. However, overactivation of C5a receptors is associated with progression of the systemic inflammatory response syndrome. While usually associated with bone marrow derived cells, massive upregulation of C3a and C5a receptors occurs on lung epithelium following exposure to LPS. The therapeutic potential of antagonizing the anaphylatoxin receptors in chronic lung diseases associated with infection, such as COPD and Cystic Fibrosis, will be discussed.

Craig Gerard, *Harvard Medical School, Boston, USA*

▷ THE ROLE OF THE NADPH OXIDASE IN THE KILLING OF BACTERIA AND FUNGI BY NEUTROPHILS - REPLACING THE PARADIGM OF FREE RADICAL DAMAGE WITH ION PUMPS AND pH OPTIMISATION

The NADPH oxidase plays an essential role in the killing of bacteria and fungi by neutrophils. Defects in this oxidase or an anaerobic environment impairs killing. The oxidase produces copious amounts of superoxide, which, together with other "reactive oxygen species" was thought to directly kill the microbes. This is not the case. The oxidase generates a charge across the wall of the phagocytic vacuole that pumps ions across the membrane. Cl⁻ is pumped out of, and K⁺ into, the vacuole which, together with activation of type 1 Na⁺/H⁺ exchanger which exchanges Na⁺ in the vacuole for H⁺ in the cytoplasm, activate the microbicidal enzymes released into the vacuole from the cytoplasmic granules.

Anthony W. Segal, *University College London, United Kingdom*

▷ DEFENSINS AND THE LUNG: PAST, PRESENT AND FUTURE

Defensins are small, cysteine-rich peptides with a long past and an intriguing future. Produced by plants, fungi, invertebrates and vertebrates, defensins are endogenous, gene-encoded antibiotics that contribute substantially to innate mucosal immunity. At least eight different defensin peptides help protect the human lungs: four beta-defensins (HBD 1-4) and four alpha defensins (HNP 1-4). We will describe their structures, summarize their antimicrobial and antiviral properties, and suggest how they work. We will introduce the fascinating theta defensins (cyclic minidefensins) of nonhuman primates, and end by discussing the prospects of using defensins as therapeutic agents in the future.

Robert I. Lehrer, *UCLA, Center for the Health Sciences, Los Angeles, USA*

▷ ANTIMICROBIAL PEPTIDES IN THE RESPIRATORY TRACT: ROLE IN INFECTION, INFLAMMATION AND IMMUNITY

The innate immune system is an essential line of defence against respiratory infections. Antimicrobial peptides that are produced by the epithelial cells lining the airways and the neutrophils are important contributors to this line of defence because they display antimicrobial activity against a range of micro-organisms. It is now recognized that these molecules not only kill inhaled bacteria, fungi and viruses, but also display other functions in immunity and wound repair by activating a range of cell types, including neutrophils, dendritic cells and epithelial cells. This talk will address these new insight into the central role of antimicrobial peptides in the lung.

Pieter S. Hiemstra, *Leiden University Medical Center, The Netherlands*

▷ GENETIC PROGRAMS REGULATING HOST DEFENSE OF THE LUNG

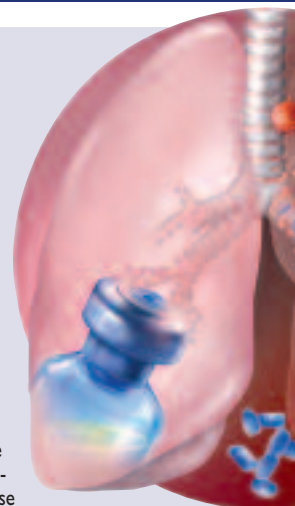
A complex transcriptional program coordinately regulates the expression of molecules mediating innate immunity and pulmonary surfactant that are required for lung homeostasis. Activation of these transcriptional programs are, in turn, influenced by signaling molecules including growth factor and cytokines that enhance cell survival and induce the synthesis of innate host defense molecules including the surfactant collectins, SP-A and SP-D.

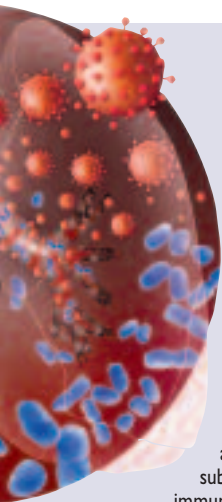
Jeffrey A. Whitsett, *Cincinnati Children's Hospital Medical Center, Cincinnati, USA*

▷ GENETIC POLYMORPHISM OF HUMAN SURFACTANT PROTEIN A AND HOST DEFENSE

In the course of evolution, the surfactant protein (SP-) A, an innate host defense molecule, acquired progressively genetic and epigenetic complexity. This may, in specific, reflect the importance of SP-A as innate host defense molecule and, in general, the need for a more diverse host defense system, as one moves up the evolutionary ladder. In the presentation, I will discuss the different types of SP-A complexity, and regulatory and functional differences among SP-A variants with focus on the differential ability of human SP-A variants to enhance phagocytosis of *P. aeruginosa* by macrophages. Mechanisms and working models may also be discussed.

Joanna Floros, *The Pennsylvania State University, Hershey, USA*





▷ CONTAGION: PERSON-TO-PERSON SPREAD OF RESPIRATORY INFECTIONS

Contagion of respiratory organisms depends upon biological factors that affect the capacity of an organism to proliferate in the airways and spread via secretions, and the size of the inoculum that leads to colonization and/or infection of another person.

There are surprising differences among organisms. Three general patterns will be discussed, and specific clinical and/or experimental examples will be provided, citing a range of organisms. *Streptococcus pneumoniae*, for example, spreads from one person to another generally by direct contact with nasopharyngeal secretions, but does not directly produce infection; rather, a stage of colonization and proliferation in the upper airways may be followed by development of infection (e.g., otitis media, sinusitis or pneumonia), with many host factors influencing the likelihood of such an occurrence. In contrast, influenza virus aerosolizes; inhalation after a single exposure may be followed immediately by infection in a substantial proportion of exposed persons if they are not specifically immune to the infecting type. Finally, *Mycobacterium tuberculosis* also aerosolizes and the infection rate may be quite high but, unlike the situation with influenza, exposure tends to be prolonged. However, most infection is subclinical, so that contagion may be undetected; disease then occurs each year in a certain proportion of those persons who have had subclinical infection. Patterns of contagion by other bacterial and viral organisms will be compared and contrasted to these three examples.

Daniel M. Musher, Baylor College of Medicine, Houston, USA

▷ NOSOCOMIAL PNEUMONIA: KILLER, OR MARKER OF SEVERITY

Nosocomial pneumonia (NP) is a frequent event in severe patients, in particular after aspiration, surgical procedures, and mechanical ventilation with intubation or tracheotomy. In this last group of patients, when intubation stays more than 3 days, incidence is up to 20 to 30%. People use the term VAP (ventilator associated Pneumonia), but it is unfortunate, since NP is due to the disease which made the intubation necessary, rather than to the tube itself. NP is considered as a very serious event, responsible for a high mortality, and possibly related to a poor quality of care. It is clear in the literature that crude mortality of this event is very high (40 to 50%), but controversies persist on the real attributable mortality of this infectious event, and on the possibility to prevent it. In some settings, the event is related to an aspiration which happened before admission in the hospital, or the ICU (severe trauma, coma of any origin, end of life...). In those cases, it is easy to understand that NP is a marker of severity, rather than a quality indicator. One issue is that we don't know how long it could take to develop NP after aspiration. It could be due to the inoculum and the host defenses. For late NP, some preventive measures showed some efficacy (Prophylactic antibiotics, semi recumbent position in the bed...). However, many NP occur although preventive measures have been taken, and do not seem to be related to quality of care. Host defenses, in particular in the lung, and the immunodepression which happens frequently after severe infections are probably risk factors, but this remains to be demonstrated.

Jean Carlet, Fondation Hôpital St Joseph, Paris, France

▷ DO CHILDHOOD RESPIRATORY INFECTIONS PREVENT, CAUSE, OR MERELY REVEAL ASTHMA?

Viral respiratory infections have long been associated with wheezing illnesses and asthma in childhood, but the nature of this relationship is debated. While on one hand, severe RSV illnesses appear to increase the risk of asthma, evidence related to the hygiene hypothesis suggests that infections can prevent asthma. Finally, one of the most difficult questions to address is whether infections really change the risk of asthma, or alternatively, are simply indicators. Although definitive resolution of these questions await the development of practical anti-viral interventions, new data will be presented to help reexamine these fundamental questions about viruses, wheezing, and asthma.

James E. Gern, University of Wisconsin, Madison, USA

▷ PATHOGENESIS OF INFLAMMATION AND INFECTION IN CYSTIC FIBROSIS

The hallmarks of the lung pathology in Cystic Fibrosis (CF), are bacterial colonization of the lung with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, associated with an exaggerated, sustained and extended inflammatory response characterized by influx of neutrophils and increased release of pro-inflammatory cytokines. This presentation will review the factors that link the basic (CFTR mutation) defect of CF to the propensity of bacterial colonisation including the dehydration of the airway surface liquid and decreased mucus transport, the increased luminal salt concentrations that inactivate the antibacterial proteins, the increased number of epithelial and mucin bacterial defense receptors and the failure of CFTR to ingest and clear bacteria. It has been also suggested that inflammation may precede infection, whether it is related to a constitutive NF- κ B abnormal expression or to a defect in the generation of endogenous anti-inflammatory lipid mediators will be discussed.

Edith Puchelle, Inserm U514, CHU Maison Blanche, Reims, France

▷ ACUTE LUNG INJURY: MECHANISMS OF INJURY FROM BACTERIAL INFECTION

Bacterial infection is the most important cause of acute lung injury and acute respiratory failure in critically ill patients. Several mechanisms mediate bacterial-induced lung injury to both the lung endothelium and the alveolar epithelium. Injury to the alveolar epithelium

appears to play a critical role in determining the severity of acute lung injury, in part because lung epithelial injury slows the resolution of alveolar edema. Novel treatments are being developed that may attenuate the severity of bacterial-induced lung injury and promote the resolution of acute respiratory failure.

Michael A. Matthay, University of California, San Francisco, USA

▷ CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) INFECTION/EXACERBATIONS

COPD is a condition characterised by several distinct phenotypes and intermittent episodes of worsening (exacerbations). Furthermore, it is recognised as an "inflammatory disease" which is central to its progression. The role of infection has been unclear because some patients have bacteria in the lower airways continuously (colonisation) and the incidence doesn't change much during exacerbations. However recent data confirms the size of the colonising load influences inflammation (1) and the load increases (2) and bacterial phenotype changes (3) during exacerbations with increased inflammation (episodes with purulent sputum). These findings may explain some of the progression of COPD.

Robert Stockley, Queen Elizabeth Hospital, Birmingham, United Kingdom

▷ TUBERCULOSIS: A SUBTLE EQUILIBRIUM BETWEEN A PATHOGEN AND ITS HOST

Tuberculosis is a complex disease caused by *M. tuberculosis*. One bacteria infects one macrophage present in one alveolus anywhere in the lung. In absence of immune response the bacteria will grow unlimited, kill the infected subject, but disappear at his death. At the opposite the immune response recruits numerous T-cells that create inflammatory foci able to restrict bacterial growth. The infection stops at this stage, 90/95% of subjects are sensitised but not ill. The bacteria will also disappear. Pulmonary tuberculosis occurs only in some subjects. A cavity appears in a given lung inflammatory focus. Rare bacteria that have persisted in quiescent form during months or years are able to grow in the necrotic tissues. The bacteria are save, able to contaminate new subjects around the diseased person. The immune response creates conditions for the species survival. *M. tuberculosis* is totally dependant of human beings and of their immune responses.

Gilles Marchal, Institut Pasteur, Paris, France

▷ MODELS OF LUNG INFECTIONS IN MICE: USE OF ADENOVIRUS VECTORS CODING FOR ANTIMICROBIAL MOLECULES FOR THERAPEUTIC AND VACCINATION STRATEGIES

Endogenous antimicrobial molecules (EAMs) are evolutionarily ancient elements of the host defense system against infections that can be found in animals, plants and bacteria. In addition to killing micro-organisms, antimicrobial molecules contribute to innate immune host defense activities (chemotactic, immunostimulatory and mitogenic). In particular, EAMs can use a variety of receptors on innate and immune eukaryotic cells to activate adaptive immunity. Recently, adenovirus vectors have been used to over-express EAMs (such as cathelicidin and elafin) in the protection of mice against lung infections (*Staphylococcus aureus*, *Pseudomonas aeruginosa*). We also propose that these molecules may be useful as potential adjuvants in vaccination strategies.

Jean-Michel Sallenave, MRC Centre for Inflammation Research, Edinburgh, United Kingdom

▷ INTERVENTION STRATEGIES FOR NEWLY EMERGING RESPIRATORY VIRUSES

In the past decades, we have been confronted with an increasing number of newly emerging virus infections, many of these are caused by respiratory viruses like SARS-CoV, avian influenza viruses and hMPV. With the advent of modern technology, our possibilities for the development of early warning systems and preparedness plans have increased significantly, allowing us to rapidly develop adequate intervention strategies. These include the use of surveillance, antiviral strategies and vaccination. An overview will be given of the strategies that are currently needed to combat these newly emerging infections efficiently.

Albert D.M.E. Osterhaus, Erasmus MC, Rotterdam, The Netherlands

▷ VIRAL-VECTORED RESPIRATORY MUCOSAL IMMUNIZATION STRATEGIES AGAINST TUBERCULOSIS

Pulmonary tuberculosis (TB) remains one of the leading infectious causes of death. While BCG vaccine is effective in controlling childhood TB, it is ineffective for adult TB. There is an urgent need to develop heterologous vaccines that are able to effectively boost and sustain the protective immunity triggered by parenteral BCG prime immunization. We have developed recombinant adenovirus- and vesicular stomatitis virus-vectored TB vaccines and our findings suggest that these viral platforms are very promising boost vaccines for BCG prime immunization in experimental models.

Zhou Xing, McMaster University, Hamilton, Canada

▷ SURFACE PROTEINS OF STREPTOCOCCUS PNEUMONIAE: THEIR ROLES IN VIRULENCE AND POTENTIAL AS VACCINES

Pneumococcal proteins may offer an alternative to the existing polysaccharide and polysaccharide-protein vaccines. If successful they should lower vaccine cost enough so that they can be used worldwide to help prevent the more than 1.4 million deaths in children and probably an equal number of deaths in the elderly. Many pneumococcal proteins have been described that are necessary for full virulence. These proteins can reduce complement deposition, modify host molecules, damage host cells, and play roles in attachment and invasion. Immunization with many of the proteins can cause significant protection in animal studies. Immunization with mixtures of them can result in complete protection.

David E. Briles, University of Alabama, Birmingham, USA