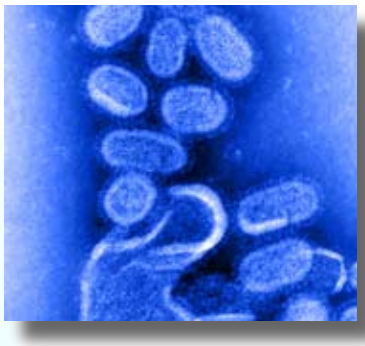
Models to Investigate the Link Between the Mucosal Immune Response in the Lung and Respiratory Tract and Disease Outcomes

Background

Humans are in constant contact with millions of microbes, including both infectious and non-infectious pathogens. The initial site of exposure, and our first line of defence, is often the mucosal immune system in the lung - a particularly important and understudied site of pathogen/host interaction. Statistics from the World Health Organization report that acute bacterial and viral lower respiratory tract infections kill more than 4 million people every year and affect millions more. Diseases such as community or hospital acquired-pneumonia, influenza, and the emergence of new infectious agents (for example SARS and avian influenza), and multi-drug-resistant pathogens highlight the importance of understanding the immune/inflammatory responses in the lung. Although the immune response in the lung plays a key role in protecting the host from infectious agents, it is also a two-sided coin where in some cases the immune/inflammatory responses are actually the cause of morbidity or even death. Examples of the adverse effects of inflammation include autoimmune disease, allergy and asthma.



In 2004, the CIHR Institute of Infection and Immunity (III), in partnership with the CIHR Institute of Circulatory and Respiratory Health, AllerGen, a Network of Centres of Excellence (NCE), and the Canadian Cystic Fibrosis Foundation (CCFF), launched a Request for Applications (RFA) entitled "Models to Investigate the Link Between the Mucosal Immune Response in the Lung and Respiratory Tract and Disease Outcomes"

This document describes some of the outcomes and research discoveries that correspond to the original five objectives of the initiative.

AllerGen

CIHR IRSC
Canadian Institutes of Health Research / Instituts de recherche en santé du Canada
Institute of Infection and Immunity / Institut des maladies infectieuses et immunitaires
Institute of Circulatory and Respiratory Health / Institut de la santé circulatoire et respiratoire

Canadian Cystic Fibrosis Foundation
Fondation canadienne de la fibrose kystique

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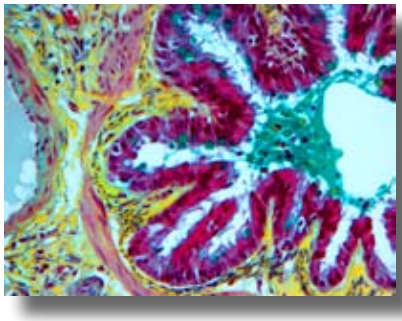
Objective #1: *To promote excellent research contributing to the advancement of knowledge in the area of mucosal immune responses in the lung and the upper respiratory tract.*

In 2006, III and partners committed $4.46 million over 3 years to support nine successful research projects on a variety of topics involving over 24 researchers from across the country. Below is a list of the funded researchers and project titles:

Principal Investigators	Institution	Project Title
BAI, Tony R; KNIGHT, Darryl A; VAN EEDEN, Stephanus	University of British Columbia, St. Paul's Hospital	Environmental impact on the epithelial immune barrier in asthma
DAY, Robert; CADIEUX, Alain; SEIDAH, Nabil G; TALBOT, Pierre J	Université de Sherbrooke	The implication of PCs in mucosal immune responses in the lung and respiratory tract. Le role des proprotéines convertases dans la réponse immunitarie muqueuse des poumons et des voies respiratoires.
DURONIO, Vincent; KHALIL, Nasreen; LEVINGS, Megan K	University of British Columbia; Vancouver Coastal Health Research Institute	The mechanistic basis of post-lung transplantation bronchiolitis obliterans and therapeutic approaches
DUSZYK, Marek; FOLEY, Edan IRVIN, Randall T; VEDERAS, John C	University of Alberta	Models of bacterial lung infection in Cystic Fibrosis: Therapy with Antimicrobial Peptides
LAVOIE, Jean-Pierre	Université de Montréal	Study of the pathogenesis and reversibility of airway damage and repair (remodeling) during chronic mucosal immune responses to environmental allergens
MCNAGNY, Kelly M; KUBES, Paul; MODY, Christopher H	University of British Columbia	Role of mast cells and eosinophils in allergic inflammation and fibrosis in the lung
PARKS, Robin J	Ottawa Health Research Institute	The Innate Immune Response to Human Adenovirus
SAD, Subash	University of Ottawa	Mechanisms of induction and maintenance of T cell memory in the lungs
XING, Zhou; GAULDIE, Jack; JORDANA, Manel; STAMPFLI, Martin R	McMaster University	Regulation of lung mucosal immune responses by heterologous exposure to multiple infectious and allergic agents

Objective #2: *To establish new experimental research models which enable the systematic investigation mucosal immune responses.*

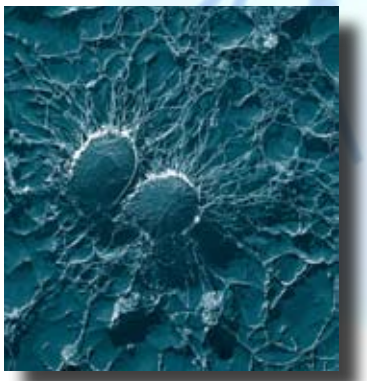
Dr. Jean-Pierre Lavoie and his team at the University of Montreal are using a disease that naturally occurs in horses to study the loss of airway function in persistent asthma. The uncontrolled immune responses to environmental antigens seen in human asthmatics eventually lead to inflammation and changes in the airways and lung parenchyma. In the long term these effects lead to decreased lung function, which may be mediated in part by a remodelling and increase in airway smooth muscle (ASM) mass. It is suspected that these changes may not be entirely reversible even following withdrawal of the allergen or by controlling inflammation through administration of inhaled corticosteroids. The equine model enabled the team to study the reversibility of airway remodelling and to correlate changes with lung function, inflammation and gene expression. As a result it was demonstrated that a three-month withdrawal of antigenic exposure did not reduce the ASM[1]. It remains to be seen whether application of inhaled corticosteroids will reverse the effect and this is the topic of ongoing studies. More than 140 genes were identified that are differentially expressed in the lung tissue of asthmatic horses[2] and it is hoped that further study of these genes will lead to the identification of novel biomarkers as therapeutic targets.



The distal airway from a horse

An endoscopic evaluation of the upper airways of a horse

Influenza Virus

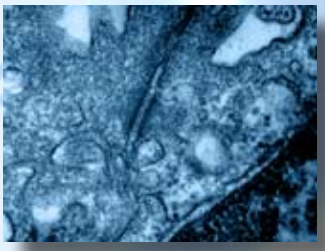


Staphylococcus

The use of animal models has contributed to our understanding of mucosal host immune responses to environmental agents such as microbes and aeroallergens. However, most studies have focused on the pathogenesis of individual agents presented in isolation, whereas in reality humans are constantly exposed simultaneously to a wide variety of environmental antigens. **Dr. Zhou Xing** and his team at McMaster University have developed a murine model for studies on complex exposures to viruses (influenza), bacteria (*Staphylococcus aureus*) and aeroallergens (house dust mite). Results from these studies have demonstrated that when a viral flu infection is followed by a bacterial infection, a situation often mimicked in real life, the natural killer (NK) immune response against the bacterial infection is significantly impaired[3]. Further experiments in which mice were initially infected with an influenza virus and then exposed to a sub-clinical dose of gram-negative bacteria, demonstrated a more severe sickness than in those infected by either infection alone. This indicates that prior flu infection compromises the host response to subsequent challenges. Interestingly, in the aeroallergen model, it was shown that allergen exposure during a flu infection results in a worsening of the allergic response whereas exposure at the end of a flu infection reduces the allergic response. These studies shed light on the complex interactions between multiple antigen exposures and host responses[4].

Objective #3: *To identify novel outcome markers of lung function, with specific reference to the immune system*

Chronic asthma eventually leads to impaired lung function, which can be exacerbated by simultaneous exposure to air pollutants and viruses. **Dr. Tony Bai** and his team at the University of British Columbia are studying airway epithelial cells grown from both asthmatic and non-asthmatic airways to explore the differences in repair kinetics following exposure to viruses and/or particulate air pollution (PM10) and have shown that injured asthmatic airway cells repair much more slowly than non-asthmatic cells, a process mediated by the cytokine IL-13[5]. Other cytokines are also involved in maintaining the immune barrier and studies show that perturbations in the release of these cytokines and the expression of cell receptors in asthmatic cells leads to increased inflammation, immune cell persistence and impaired repair and remodelling[6]. Further studies on basal cell biomarkers have demonstrated that the changes in airway epithelium happen early in the disease process and results in an epithelium that is unable to form an appropriate immune barrier[7].



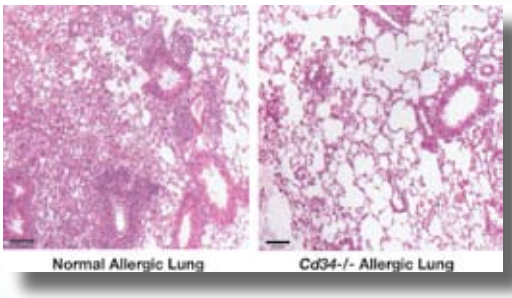
airway epithelial cells

Dr. Vincent Duronio and his team, also at the University of British Columbia, are studying the reasons for failure in lung transplantation. While frequently the only option for many lung diseases, lung transplants can eventually fail because of a build up of tissue (fibrosis) in the airways. Known as post lung transplant bronchiolotis obliterans (PLT-BO), this common sequelae is seen in up to 90% of transplanted lungs by nine years post transplant. Dr. Duronio's team has successfully established a novel mouse model system in which to study PLT-BO, something that did not exist in Western Canada prior to this grant and using this system has been able to demonstrate that by blocking apoptosis (cell death) with viral vectors, airway fibrosis could be prevented. These findings represent a new approach in preventing lung rejection. Additional studies on a peptide, previously patented by the group, that can block the action of a potent pro-fibrotic cytokine, show that long term prevention of fibrosis in the transplanted graft is possible, suggesting another potential therapeutic approach in the prevention of lung failure and rejection.

Murine BO: Heterotopic Trachea Transplant

Objective #4: To study pathogenesis of inflammation caused by allergens or infectious agents in the lung and upper respiratory tract

Allergy, asthma and hypersensitivity pneumonitis are inflammatory lung diseases that affect 1 in 4 Canadians with varying degrees of severity. These diseases, although seldom fatal, have a significant impact on quality of life and lost productivity. **Dr. Kelly McNagny** and his team at the University of British Columbia are studying the role of two important cells types: mast cells and eosinophils in inflammation, tissue damage and disease progression. The team used a fluorescent tag to monitor the generation and migration of mast cells and eosinophils into the lungs of living mice. Their results have shown that there are yet-to-be-discovered sensors of pathogen infection in the lung as none of the major known innate immune factors are effective in a zymosan-induced lung inflammation model system[8]. Further research will be required to follow up on this intriguing observation. The group has identified a biomarker, CD34, which is expressed by mast cells and eosinophils and is required for allergic inflammation[9]. CD34 may prove to be a good therapeutic target as inactivation of the associated gene leads to resistance in mice.

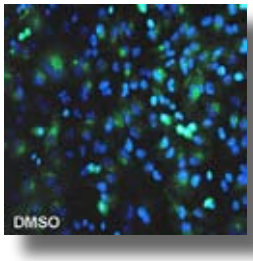


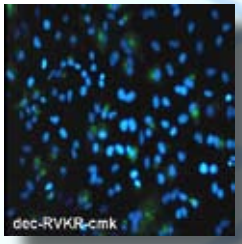
Some pathogens, such as *Mycobacterium tuberculosis* (*M.tb*), are able to evade both the innate and acquired immune systems to cause chronic infection in the host. The lung in particular appears to offer a protected site where *M.tb* can grow even in the presence of an effective systemic immune response. Many bacterial infections can be prevented through vaccination. In most cases the vaccine works by generating numerous memory T cells with specificity for the bacterium in question. These T-cells are then able to eradicate the infection and provide future protection for the host. In the case of *M.tb*, however, **Dr. Subash Sad** and his group at the National Research Council and the University of Ottawa has demonstrated that no amount of mycobacteria-specific T cells will eradicate the bacterium from the lungs, although these cells do not appear to have any functional defects[10]. The explanation appears to be the location of the bacterium, deep in the intracellular phagosomes, and its slow doubling rate. The result is that very little antigen is available for T cell activation. This suggests that a different approach will be required – one that uses the innate immune system that does not rely on T cell activation[11]. This could pose problems for individuals with impaired innate immunity such as the very young, the very old and individuals who are pregnant or sick. Another finding from the studies indicates that *M.tb* infections cause erosion of existing memory T cells, leaving the host exposed to other virulent pathogens[12]. Interferon-gamma has been shown to be the mediating factor in this T cell erosion and so it is possible that regulation of interferon-gamma expression may help control the deleterious effects of inflammation caused by *M.tb* and perhaps other agents.

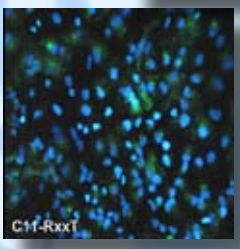
Mycobacterium tuberculosis

Objective #5: To define novel targets for prevention and therapy of immune-related lung disease

The project of **Dr. Robert Day** and his team at l' Université de Sherbrooke focused on the potential of the enzymes, proprotein convertases (PCs), as therapeutic targets in lung infections and allergic reactions. The group studied a coronavirus mouse model and also a model of allergic airway sensitization. In both cases, PCs were shown to play a critical role in disease progression and PC inhibitors were shown to be effective therapeutic agents.

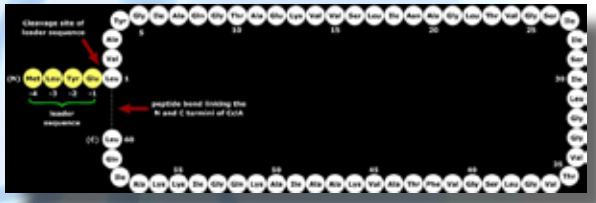






Cell-to-cell propagation of the human coronavirus OC43 is significantly blocked by the use of the PC inhibitors C11-RxxT and dec-RVKR-cmk compared to control DMSO as revealed by less green cells (presence of virus).

Using a Drosophila model to probe innate immunity, **Dr. Marek Duszyk** and his group at the University of Alberta looked at the potential of antimicrobial peptides in the treatment of lung infections in cystic fibrosis. The group identified an antimicrobial peptide, Carnocyclin A, which is capable of forming ion channels in lipid membranes that are selective for chloride. This is an important finding as the epithelial cells in cystic fibrosis patients lack or contain a dysfunctional CFTR chloride channel. Carnocyclin A, therefore, may provide an alternative mechanism for chlorine secretion and help alleviate the chronic inflammation in cystic fibrosis patients. Some antibiotic peptides produced by bacteria, although highly effective against gram positive organisms, have no activity against gram negative bacteria such as *Pseudomonas aeruginosa* (PA), a common pathogen in cystic fibrosis. The team is investigating a "Trojan Horse" approach by attaching the peptide to a normal ion transported in the hope that it will gain entry through the bacterial cell wall. Finally, Dr Duszyk's team is exploring the mechanisms underlying the interactions between PA pili and human bronchial epithelial cells to gain an understanding of the role of PA in mucociliary clearance.



Carnocyclin A

The research teams described above continue to investigate the links between the mucosal immune response in the lung and disease outcomes and many have received additional CIHR funding in this area, including **Dr. Zhou Xing** and his team at McMaster University who received a CIHR Team Grant in Mucosal Innate Immunity. In addition, this funding initiative resulted in the training of **Dr. Margaret Kelly**, a clinical hematopathologist, and her transition into basic research. Dr. Kelly (a member of Dr. McNagny's team) has been reappointed as a full faculty member in the Department of Pathology & Laboratory Medicine at the University of Calgary and is now specializing in pulmonary pathology and investigating new screening methods for lung cancer.

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