Dr Simon Rousseau tells International Innovation how recently discovered links between bacterial lung infections and cystic fibrosis can help to develop more effective treatments for chronic inflammation.

What is the potential benefit of investigating why the host defence response is deregulated in a cystic fibrosis (CF) patient’s lung?

Although great progress has been made in trying to target the underlying genetic defect in CF, for the great majority of individuals with the disorder, there are still no drugs that can effectively correct it. The challenges have been made all the more evident by elegant work showing that the most common mutation responsible for CF leads to multiple defects of the protein that all need to be corrected. There are many consequences of the loss of CFTR function (the gene mutated in CF), the most dramatic being progressive lung tissue destruction. It is therefore imperative to understand why this is occurring.

From many years of study, it has been apparent that the presence of chronic infection in the lungs of CF patients is detrimental and an important contributing factor to tissue destruction. Discovering the molecular mechanisms linking the absence of CFTR to susceptibility to infection could provide alternative strategies to delay or even prevent lung tissue destruction. This would have a huge impact on the health of CF patients.

In excess of the bacterial burden, how is inflammation a major cause of morbidity and mortality in CF patients?

Over time, excessive inflammation leads to lung tissue destruction. These repeated bouts of inflammation eat away and obstruct the airways, impairing lung function. Towards the end stage of the disease, the only current option is transplantation as the lungs are no longer able to provide sufficient support for breathing.

Can you discuss your most recent findings on the hyper-activation of key signals that lead to inflammation in CF patients?

We discovered that the response to infection is heightened in airway epithelial cells expressing the mutant protein responsible for CF. Not only are CF lungs exposed to chronic infection but they also respond to this infection more strongly. The combination of these factors leads to excessive inflammation in relation to bacterial burden and lung tissue destruction. We are currently investigating the molecular mechanisms linking the mutated CFTR protein responsible for CF and the heightened response to infections.

The molecular mechanisms underlying the complex process of inflammation are only now becoming apparent. What have
been the major challenges to studying this process and how have they been overcome?

The major hurdle in inflammation research had been the lack of information on some of the key molecules responsible for the detection of pathogens. A lot of emphasis in medical research was on adaptive immunity (antibody-mediated immunity), a response which evolved much more recently. However, pioneering work from Bruce Beutler and Jules Hoffman led to the discovery of pathogen sensors and helped kick-start the current interest in inflammation research.

What do you hope will be the medical impact of better understanding the mechanisms underlying inflammation?

A good understanding of the fundamental mechanisms regulating any physiological process, such as inflammation, is important to improve treatments and generate novel drugs. To be efficient, inflammation must be well balanced. In cases where this balance is tipped one way or another, we need ways to push it back in the right direction.

Contrary to some other areas of medical research, in most cases it may be interesting to affect the response by a smaller margin than a complete inhibition as it may minimise unwanted effects. Therefore, a good basic understanding of the signals regulating inflammation will give us clues on how to correct the response when it is perturbed.

FIGHTING CYSTIC FIBROSIS (CF), the Cystic Fibrosis Strategic Research Group (CFSRG) is dedicated to developing new therapies specifically aimed at treating the disorder’s effects on the respiratory system. The group is formed by investigators from four universities and six CF paediatric and adult clinics. By integrating work from top university medical centres and clinics in Quebec, the Group has created a wealth of shared resources with which to address the problem of compromised upper airways; the primary cause of mortality and morbidity in patients suffering from CF.

Created under the Fonds de Recherche Québec Santé (FRQS), the Strategic Research Groups of the Respiratory Health Network (RHN) marry the various specialisms of researchers, pulmonologists and other health professionals including experts from Laval University, the University of Montreal, McGill University and the University of Sherbrooke. Through this professional base, RHN aims to promote the integration of clinical, evaluative, epidemiological and fundamental research into projects that can make a significant impact in addressing issues of respiratory health. Connecting these scientific fields and directing activities toward common goals attracts more researchers, maximising the interdisciplinarity at its core.

TODAY’S TARGETS

Patients currently suffering the most severe effects of CF are faced with one course of action: lung transplantation. With the threat of organ rejection a constant concern, CFSRG is committed to improving the options for patients but today, treatment of respiratory CF is a considerable challenge. Already fraught with complexity, the burden of tackling respiratory CF has grown since 40 per cent of adults with the disease have been tested positive for diabetes. With the pooled knowledge and resources that comes from connecting various scientific backgrounds, however, CFSRG’s work provides invaluable access to research expertise in all CF-related areas.

Another major advantage of sharing resources is the access it enables to clinical materials such as bacterial strains, mucus and blood samples that facilitate the potential development of novel therapies. As a network of vital importance to investigators, CFSRG has for the last four years supported an initiative to supply primary bronchial airway epithelial cells from transplanted CF lungs.

The Group reacts quickly to new developments in treating CF. Now it has become clear that a healthy microbiome in humans is important for health overall, CFSRG has embarked on a new project focusing on the characterisation and impact of the bacterial community living in the lungs. Investigations are underway to study the composition of the lung microbiome of CF patients and its impact on CF lung disease as they look to demonstrate that alterations and a loss diversity of the lung flora may lead to poorer defenses against lung pathogens and contribute to worsening of the disease.

PROMOTING INTEGRATION

CFSRG’s activities are directly relevant to real-world challenges, and place emphasis on putting patients first. Further projects include looking at novel nutritional supplementation to improve overall health; the mechanisms underlying CF-related diabetes, a very important co-morbidity in the adult CF population; and ways to re-establish the overall inflammatory balance: decreasing inflammation while preserving host defense mechanisms. Other investigators are also looking at therapeutic aimed at the basic molecular defect, correcting the most common mutation found in CF.

Such a high level of collaboration between institutes is hugely important as access to patient-derived samples is key to making possible the design of larger scale clinical studies and arriving at a better understanding of this disease. The interdisciplinarity of the CF strategic research group is geared toward one purpose only: facilitating new ideas and discoveries to improve the health of patients with CF.
Illuminating inflammation

Chronic inflammation is the dominant pathogenic feature of lung disease in patients with cystic fibrosis, yet surprisingly little is known about its underlying molecular mechanisms. However, new developments in dynamic imaging techniques at McGill University, Canada are set to change the situation.

INFLAMMATION IS AN essential part of the body’s defence mechanism against infectious agents and, as an easily observable response, its importance has been recognised for centuries. However, if inflammation is prolonged and cannot resolve itself then it can lead to asthma, cystic fibrosis (CF), chronic obstructive pulmonary diseases (COPD) and lung cancer. Anti-inflammatory medicine has often been a first port of call for illness and injury, however, there is growing concern that not only may this approach be unnecessary but it could be even be harmful in the long term as bypassing the initial stages of recovery may blunt the body’s response to stress.

To understand a host’s defence mechanisms more fully it is necessary to study the intracellular networks activated in response to infection. Research in this area has, however, been held back by the standard probing tools available in the field. Currently, with only the ability to observe the assembly and operation of these intracellular networks at specific moments in time, the data collected has been disjointed and incomplete. Obtaining a truly comprehensive picture of the molecular mechanisms of host-pathogen interactions requires novel methods of continuous monitoring.

RESPONDING TO INFECTION
Dr Simon Rousseau, an assistant professor at McGill University’s Department of Medicine, Montreal is currently developing fluorescent sensors to study host-pathogen interactions in rare genetic disorders. Rousseau, who is also one of 22 research directors at the University’s Meakins-Christie Laboratories, aims to build a clearer picture of the intracellular signalling pathways activated during host defence response: “Our main goal is to create novel tools that enable us to study the regulation of specific molecules within these intracellular networks in real-time, filling those missing gaps,” he states.

When a host responds to bacterial or fungal infection, the first line of defence is the recruitment of neutrophils – white blood cells answering the distress signals emitted when a pathogen is first detected. Careful regulation of neutrophilic inflammation is important for the host to maintain a healthy response to the pathogen and avoid a progression to chronic inflammation and its associated risks. Models of neutrophilic inflammation triggered by epithelial infection in Rousseau’s lab have shown three pathways in particular as responsible for coordinating the synthesis of inflammatory mediators. However, the current state of cell signal imaging means that variables such as amplitude, oscillation and duration are usually missing from the equation, making further analysis difficult. Exploiting the advances over the last 20 years in sensor technologies, Rousseau is developing bioluminescence resonance energy transfer (BRET) probes and fluorescence complementation assays to pick up and identify which molecules are responsible for regulating inflammation.

Before Rousseau can put the fluorescent-based probes to use, they require validation using human embryonic kidney cells. Once complete, the probes can be used to study the molecular mechanisms of infection in the epithelial cells that line the upper airways. As the first line of defence against pathogens inhaled by the host, it is here that distress signals are first emitted.

If all goes according to plan, these probes will be able to image cellular signalling during an infection in real-time: “We will be able to determine the timing and magnitude of activation of a particular signalling molecule when these cells are infected with pathogenic bacteria,” states Rousseau, “marking a significant advance for biomedical research in this field.”

DRUG DEVELOPMENT
Aggressive destruction of lung tissue is the primary factor causing mortality and morbidity among individuals with CF. Against this context, Rousseau’s team is striving to elucidate the underlying molecular mechanisms. For this...

Phosphorylation of key components of stress-activated signalling pathways in human cystic fibrosis lung biopsy.
Rousseau aims to build a clearer picture of the intracellular signalling pathways activated during host defence response.

Purpose, investigations have focused on dynamic imaging of intracellular signalling in hosts with CF and Niemann-Pick disease type A/B – both rare genetic disorders whose fatalities are associated with bacterial lung infections. After recreating the genetic defects at the heart of these disorders, Rousseau’s team analysed their response to bacterial infections to define what differs from their non-diseased counterparts.

From this comparison, Rousseau has demonstrated that hyper-activation of the signals that induce neutrophilic inflammation occurs in the airway epithelial cells of CF lungs, making it the primary pathological feature of CF lung disease. It was shown that upon infection, activation of the signalling pathways responsible for coordinating the synthesis of inflammatory mediators increased, as did cytokine synthesis and neutrophilic recruitment in the absence of CF transmembrane conductance regulator (CFTR). Knowing that these pathways cause destructive inflammation in the gut, Rousseau has theorised that a lack of CFTR enhances activation of the pathways, meaning more pro-inflammatory cytokine synthesis, neutrophil recruitment and inflammation.

The next step in exploring this theory of excessive inflammation in CF lung disease lies in defining the mechanisms linking the absence of functional CFTR and hyper-activation. Implementing the probes developed in his lab, Rousseau hopes to identify the signalling pathways responsible for decreasing inflammation in order to develop novel therapies further down the line. Treatments for reducing inflammation are currently available, but the benefits are often outweighed by the long list of shortcomings. For example, alongside other serious side effects, corticosteroids can make patients more susceptible to infection by suppressing the body’s natural immune response and reduce growth in children – a serious concern in paediatric diseases. Rousseau’s research aims to facilitate the development of drugs that reduce inflammation but preserve host defence, potentially making future treatment of non-resolving inflammation in CF patients considerably safer.

A MORE COMPLEX SYSTEM

With the main body of investigations focused on isolated cells models, Rousseau is currently aiming to further validate his results in more complex models on infection. A fully sequenced genome and the opportunities for visual observation afforded by its transparency and postpartum embryonic development makes the zebrafish a great model for in vivo testing with the advantage of minimum physical intervention. This means that the behaviour of fluorescently labelled immune cells recruited at the time of infection can be monitored throughout the entire organism. “This is a powerful method for identifying the signals required for regulating neutrophil recruitment to the site of infection,” states Rousseau, and one that could lead to a new generation of therapies treating chronic inflammation.

The tools developed in Rousseau’s lab will help characterise the molecular mechanisms of this condition in a way that has been impossible until now. With them, a greater understanding can be achieved and the development of drugs that preserve host defence far more likely.