DR NATHALIE GRANDVAUX

Investigating immunity

The regulation of the body's immune antiviral response remains largely unknown. **Dr Nathalie Grandvaux**'s research will further understanding of these complex mechanisms, with a focus on respiratory viruses

Could you outline your laboratory's primary research interests and objectives?

The main aim of my research group is to define the mechanisms that allow host cells to mount a very early defence response, known as the innate immune antiviral response, against invading viruses. This response is aimed at restricting viral replication and spreading before the professional immune system is engaged. Our research focuses on the molecular mechanisms that allow the very first cells to be infected upon viral invasion to trigger the gene expression encode proteins which restrict viral replication and spread and alert surrounding cells and the professional immune system to the infection. A fascinating aspect of our research is to characterise how the use of oxygen by specific enzymes in the cells to generate reactive oxygen species (ROS), including free radicals and hydrogen peroxide, constitutes a key switch that permits stringent regulation of these antiviral responses.

How has understanding of the molecular mechanisms involved in antiviral response evolved over recent years?

Surprisingly, the main antiviral molecules – the interferons – which belong to the class of cytokines carrying messages between cells, were discovered over 50 years ago. However, until the beginning of the 21st Century, our knowledge was limited to three transcription factor families that are involved in interferon gene regulation during the antiviral response. Over the past decade, the scientific community has made important breakthroughs following the identification of molecular sensors capable of detecting viruses. Since their discovery, the characterisation of the intracellular cascade that they initiate, to activate transcription factors and interferon gene expression, is expanding very rapidly. Knowledge of these mechanisms has also allowed various teams to characterise the processes employed by viruses to evade the antiviral response, an important aspect of research in the identification of novel antiviral therapeutic targets. The era of genomics also provided the tools to identify the genes that are induced by interferons and possess antiviral activities.

What have been your team's most exciting or significant discoveries to date?

Our group has identified two enzymes of the NADPH oxidase family, NOX2 and DUOX2, that are responsible for the production of ROS in the airway epithelium infected with respiratory syncytial virus (RSV) or the closely related sendai virus. Our data highlights the intricate temporal mechanisms by which different ROS-producing enzymes participate in the antiviral response. Importantly, while reversible oxidation of proteins is key to regulating cellular processes, irreversible oxidation also occurs and damages proteins. Irreversible oxidation of proteins could have long-term consequences and is suggested to be involved in numerous chronic diseases, as well as in ageing.

Are you collaborating with other projects or laboratories in the course of your investigations? Has a multidisciplinary approach proved important to the success of the project?

Succeeding in our various goals requires a collective research effort bringing together biochemists, physiologists and clinicians. Clinicians are key collaborators in order to strengthen our findings with patient samples and evaluate novel biomarkers based on our findings. Our work on airway epithelial cells has benefited strongly from our collaboration with physiologists. With the help of our clinician collaborators we expect to compare the behaviour of cells originating from both children, which are particularly relevant to RSV infection, and adults.

The identification of oxidised proteins was until recently limited by the lack of appropriate techniques. It was a challenge to study protein oxidation without introducing

a bias through the exposure of samples to oxygen when manipulating them. We are now starting to use a technique developed by one of our collaborators to detect reversible protein oxidation.

Do you have plans for future research projects or new avenues of investigation?

The primary future challenge of our work, on the redox-dependent regulation of the innate immune antiviral response, is to identify the specific oxidation of proteins and the impact on their activities. Besides the direct role of ROS, we are very interested in determining other molecular mechanisms that define the duration of the antiviral response. As mentioned above, an inappropriate innate immune response is associated with the development of various autoimmune and chronic inflammatory diseases. Although the mechanisms that initiate the antiviral response are widely studied, I believe that understanding how the response is terminated is key for the design of therapeutic targets. One of our current goals is to identify novel positive and negative mechanisms that control the duration of the innate antiviral response.

Going viral

In the quest to discover more about antiviral response mechanisms, the Hospital Research Centre at the **University of Montreal** is conducting research that could contribute to new treatments for respiratory viruses

THE BODY'S INNATE immune response system is a complex and essential early defence against viruses. The past decade has seen the discovery of specific signalling cascades leading to the secretion of proteins called cytokines, mainly interferons, which are key players in antiviral response mechanisms. Interferon-activated cells mount this response through the expression of hundreds of genes that have the capacity to restrict viral replication in infected and neighbouring cells. Proinflammatory cytokines regulated by intracellular networks, similar to the ones that trigger antiviral interferon production, also orchestrate the recruitment and activation of the cells of the professional immune system. This proinflammatory response is key to eliminating infected cells.

The initial wave of antiviral and proinflammatory cytokines is produced by the first virus permissive cells encountered by the virus. However, knowing the identity of these proteins is often not enough to understand how the innate antiviral response is appropriately regulated. If the host is unable to mount an efficient and sustained antiviral response, it fails to eradicate the infection. Conversely, an excessive innate antiviral response, in terms of intensity or duration, is associated with the development of various autoimmune and chronic inflammatory diseases. There is still a long way to go before scientists completely understand the regulation of the complex intracellular networks that control the antiviral response.

RESPIRATORY SYNCYTIAL VIRUS

As the Canada Research Chair in Signalling Viral Infections and Oncogenesis, Dr Nathalie Grandvaux has devoted her career to characterising the host response to viral infections. Based at the University of Montreal Hospital Research Centre (CRCHUM), her group is currently investigating the cellular mechanisms involved in respiratory viruses. They are particularly interested in respiratory syncytial virus (RSV), which is the major cause of respiratory illnesses like bronchiolitis and pneumonia in children, resulting in substantial morbidity and mortality. The team is aiming to elucidate the signals that allow cells to inform the genome of the viral presence, how the signals are regulated and how viruses circumvent these responses.

The group's studies focus on the airway epithelial cells; the primary target cells for respiratory viruses. "These cells orchestrate a number of key responses that are not only aimed at restricting virus penetration and replication, but also at activating the subsequent immune response," Grandvaux explains. "Thus, I strongly believe that the first interaction between the host and the virus is a key determinant in the success of the fight against the virus. The interaction of a virus with the primary target cells is the first event in a chain that needs to be accurate to reach the appropriate response."

REACTIVE OXYGEN SPECIES

Grandvaux's group has recently highlighted that there is an extra phase that needs to be added to the two phase cytokine model described above. "We showed that the cooperation of different cytokines trigger's a delayed phase that is essential to providing a sustained response. Obviously this phase needs to be carefully regulated to prevent inappropriate duration of the antiviral response," she adds. Their investigations include one of the switches that permit this regulation: the use of oxygen by specific enzymes to control the antiviral response triggered by the respiratory virus in airway epithelial cells.

Oxygen derivatives, or reactive oxygen species (ROS), derive from oxygen metabolism and

CANADA RESEARCH CHAIR IN SIGNALLING VIRAL INFECTIONS AND ONCOGENESIS

OBJECTIVES

To characterise the host response to virus infections, particularly in the airway epithelial cells, which are the primary target for respiratory viruses. These cells orchestrate a number of key responses that are not only aimed at restricting virus penetration and replication, but also at activating the subsequent immune response. The research focuses on the molecular mechanisms that allow the cells to trigger the expression of genes encoding molecules with antiviral or proinflammatory activities in response to respiratory virus recognition.

KEY COLLABORATORS

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NATHALIE GRANDVAUX received an Engineering degree from the French engineering university at the National Institute for Applied Sciences (INSA), Lyon and a PhD from Joseph Fourier University in Grenoble, France where she studied the structure and function of proteins involved in the generation of free radicals. During her postdoctoral studies at McGill University, Canada, she studied the mechanisms of the host defence against virus infection. She is now a Principal Investigator at the University of Montreal Hospital Research Centre (CRCHUM) and an Associate Professor in the Department of Biochemistry. She holds the Canada Research Chair in Signalling Viral Infections and Oncogenesis.

include highly chemically reactive free radicals, such as superoxide, and non-radicals, such as hydrogen peroxide; species that are largely considered to be toxic. "It is true that when produced excessively or in an inappropriate fashion, ROS contribute to oxidative stress and processes such as lipid, protein and DNA damage, thus playing a central role in the pathogenesis of various diseases, including lung diseases. However, ROS produced at lower levels, in response to specific stimuli at specific locations, are key regulators of many physiological processes," Grandvaux reveals. Oxygen derivatives act on cellular proteins through the reversible oxidation of key amino acids, mainly thiol residues, thereby regulating their activities. "This field of redox biology is becoming more and more fascinating and is currently undergoing a major change, with the development of new techniques that have begun to allow us to detect oxidised proteins," notes Grandvaux.

BREAST CANCER

Another more recent focus of Grandvaux's research is related to oncogenesis processes in breast cancer, the second highest cause of mortality among women. Her group's interest in the disease was derived directly from their investigations into the antiviral response. "In 2003, we found that the IKKe kinase plays a central role in the innate antiviral response through phosphorylation of the IRF3 transcription factor, a step necessary for interferon production," Grandvaux elaborates. "More recently, we observed that IKKe expression during virus infection is dependent on ROS production. Interestingly, IKKe was also shown to contribute to breast cancer cells proliferation. Uncontrolled ROS production in various cancers have long been shown to contribute to oncogenesis. Thus, targeting ROS could be an interesting strategy to decrease cancer cell proliferation."

NEW THERAPEUTIC TARGETS

In their work so far, Grandvaux's group has identified two enzymes that are responsible for the production of ROS in the airway epithelium. ROS derived from these two enzymes are required to develop an efficient innate antiviral response. While one of these enzymes is required for the cell to initiate the antiviral response, the second enzyme is part of a delayed phase that allows the cytokine-mediated antiviral response to be sustained. Not only have these findings contributed to understanding of the antiviral response, they may also have a significant impact on the elucidation of host-response-associated damage and long-term consequences, including ageing. "Whether oxidation of proteins is involved in virus-associated diseases or ageing of the immune system remains to be determined and will be one of the next challenges in the field," Grandvaux notes.

The results of Grandvaux's innovative research will also contribute to identifying new therapeutic targets for the treatment of RSV and breast cancer. "We hope that the identification of the sources of ROS and their targets will allow the development of more specific therapies to modulate the fate of the innate immune antiviral response," she enthuses. "Ideally, a therapeutic strategy should conserve or improve the antiviral arm and/or decrease the inflammatory response. Alternatively, identified oxidised proteins could become appropriate biomarkers for chronic inflammatory diseases or autoimmune diseases."

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