Body’s little helpers

Professor Carola G Vinuesa recalls her academic route into immunology research, and how this background has led her into the study of certain cells integral to the antibody response.

Some of the most important findings were independently reported by different teams, but each of the publications was the result of multiple collaborations between several groups. In our case, we could not have made our discoveries without important input from researchers in the UK (Dr Facundo Batista, London Research Institute; Professor Ken Smith and Dr Michelle Linterman, University of Cambridge), Belgium (Associate Professor Adrian Liston, University of Leuven), the US (Professor Pamela Schwartzberg, National Institutes of Health) and Japan (Associate Professor Sidonia Fagarasan, RIKEN) besides many Australian collaborators.

Could you explain how the T follicular regulatory (Tfr) cell may be key to understanding negative selection of self-reactive B cells?

For a long time, there has not been a satisfying mechanism to explain how those germinal centre B cells that become self-reactive during the process of somatic hyper-mutation are eliminated. Although this mutation process is required for some B cells to acquire higher affinity for the infectious organism, it is well-known that its random nature also allows the emergence of self-reactive clones. B cells can also fool T cells by presenting foreign antigens unrelated to the antigen their antibody products recognise. Our discovery of Tfr cells in germinal centres that eliminate germinal centre B cells that do not bind the immunising antigen offers the first plausible mechanism of negative selection.

You made the landmark discovery that excessive production of T follicular helper (Tfh) cells, due to a mutation in the Roquin gene, lowered the threshold of selection of mutated B cells in germinal centres, allowing the escape of cells that had become self-reactive in the process. How might this finding shape future research?

This discovery placed Tfh cells on the map of systemic autoimmunity and identified control of Tfh numbers as a key tolerance checkpoint to prevent the emergence of autoimmunity diseases in which antibodies are pathogenic or act as disease triggers. Since that key study, we and others have reported that disregulated Tfh expansion or function causes or is likely to significantly contribute to human diseases including systemic lupus erythematosus, rheumatoid arthritis, Type 1 diabetes, juvenile dermatomyositis, etc. This has sparked interest not only in using circulating Tfh cells in the blood as biomarkers of disease pathway and disease activity, but also in developing new therapeutics that specifically target Tfh cells for the treatment of these autoimmune diseases.

Are you in conversation with pharmaceutical companies to develop new drugs that dampen Tfh activity?

We have transferred our lupus mouse models to companies like Medimmune, interested in developing therapeutics targeting Tfh cells by inhibition of Tfh effector molecules such as ICOS. We have also recently described how the cytokine IFN-γ promotes autoimmunity by causing Tfh cell accumulation; this finding may refine the biological readouts of ongoing trials using antibodies against IFN-γ in lupus. In addition, recent new insights into how to modulate the Tfh:Tfr ratio may also be amenable to pharmacological manipulation, so that is an area we are going to focus on.

What do you hope your continued research in this direction will uncover in the future?

Ultimately, we would like to elucidate the key Tfh-derived signals that determine formation of high-affinity and non-self-reactive memory B cells in germinal centres. This will contribute to the development of successful vaccines for HIV, malaria and TB, and to more effective treatments for autoimmune diseases and immunodeficiencies. Tfh cells also appear to be important chronic reservoirs of HIV virus; understanding Tfh cell homeostasis may also offer the possibility of developing a permanent cure that eradicates HIV completely.
Helping fight infection

The Humoral Immunity and Autoimmunity Group from the Australian National University has investigated the role of T follicular helper cells in the immune response of B cells against infection.

**B CELLS ARE** a form of white blood cell that are an essential element of the immune system. Primarily, the role of B cells is to manufacture antibodies against antigens and to trigger T cell transformation to fight infection. They are also a precursor to memory B cells, which form upon first infection and can persist throughout life so that the immune system can produce a strong and specific antibody response upon reinfection.

The Humoral Immunity and Autoimmunity Group, led by Professor Carola G Vinuesa from the Australian National University (ANU), aims to understand the signals that drive ordinary B cells to become memory B cells, recognising and responding to infections by producing high-quality (‘high-affinity’) antibodies. Their research has proven that the efficiency of the response is directly related to the type of help the B cells receive from T cells.

Understanding how helper T cells are regulated and how these high-affinity memory B cells are generated in response to infection could prevent a range of infections including HIV and malaria, and cure autoimmune diseases such as rheumatoid arthritis and lupus. The discovery of different types of follicular helper T cells has paved the way to understanding how the strength, reactivity and quality control of an antibody response is determined.

**GERMINAL CENTRES**

While this knowledge is crucial to the development of safe and effective treatment and prevention of autoimmune and infectious disorders, its complexity has made it consistently difficult to decipher until now. “Generation of memory B cells takes place in highly specialised microenvironments called germinal centres that only form transiently in the spleen and lymph nodes,” explains Vinuesa. “Unlike other immune responses that can be investigated in a Petri dish, germinal centre reactions cannot be mimicked in the laboratory – they need to be investigated as they occur in vivo.”

The research also poses a challenge due to the nature of the cells that assist B cells in reaching this special state. Thanks to recent advances in genomics and the establishment of a repository of samples from patients with autoimmunity, in parallel with novel mouse models of these diseases, the ANU group has now identified a subset of T cells known as T follicular helpers (Tfh), which aid the B cells in germinal centres and, when deregulated, cause autoimmunity. The first insights came from their discovery that excessive production of Tfh cells, caused by a mutation of the Roquin gene, allowed the escape of self-reactive B cells. This was due to a lowered selection threshold of mutated cells, and led to a groundbreaking paper published in *Nature*.

It is hoped that elucidating how Tfh cells are regulated will lead to even further breakthroughs in understanding the development of memory B cells and autoimmune diseases.

**T CELL PHENOTYPES**

Recognising the existence of different functional types of Tfh cells occurred as Vinuesa and her colleagues studied their phenotypic appearance within germinal centres. They noticed that they were more heterogeneous than previously thought, providing a clue to their specialist role, and built their hypothesis based around their observations: “Some expressed markers that were only previously found in regulatory T cells such as Foxp3, or markers typical of natural killer T cells (NKT) such as NK1.1,” highlights Vinuesa.

As they studied the germinal centres, they identified a unique differentiation pathway centred around expression of the transcription factor ‘BCL6’ and interaction with B cells that allowed the CD4+ T cells to enter the germinal cells. The team then demonstrated that this pathway was shared by all other T cell subsets.
present in the germinal centre, requiring the same sophisticated sequence of molecular interactions and signals, which explained how the selection process allowed entry to both regulatory T cells (Tregs) and NKT cells.

Instead of helping B cells with bound protein antigen, NKT cells help B cells with bound lipid antigen. This is the first demonstration that NKT can control a germinal centre, but unlike the T cells that induce germinal centres to protein antigens, Vinuesa and her colleagues discovered that NKT follicular helper (NKTfh) cells do not generate memory B cells or long-lasting plasma cells, despite inducing a fast, strong antibody response. This is possibly to prevent the generation of long-lived B cells with autoreactivities, given the similarities between some microbial and self glycolipids.

**TFH SUBSETS**

The ANU team’s work has led to the identification of four different Tfh types, each of which plays a small but crucial role when triggering an immune response.

- **‘Pre-Tfh’** cells prime B cells for differentiation into both germinal centre B cells and extrafollicular plasma cells – the latter form and die rapidly and produce low-affinity antibodies. Pre-Tfh cells can be the precursor of GC Tfh cells, and localise to the border between B cell follicles and T zones. These cells express the key identifiers of Tfh cells, such as Bcl-6, CXCR5 and PD-1, but in lower quantities.

- **‘GC-Tfh’** cells are the Tfh cells found inside germinal centres, which select mutated B cells to survive and become long-lasting memory B cells.

- **‘NK-Tfh’**, or natural killer T cells, help lipid-binding B cells begin to produce an antibody response.

- **‘Tfr’**, or T follicular regulatory cells, are a regulatory cell type which dampen the numbers of Tfh and germinal centre B cells. Although they share many phenotypic markers with standard Tfh cells, their behaviour is very different. The researchers believe the dampening effect may be key to prevent germinal centre-derived autoimmunity, and could provide an insight into negative selection of self-reactive B cells.

Autoreactive B cells will produce autoantibodies that are the root cause of autoimmune diseases such as lupus and can trigger Type 1 diabetes. Therefore, understanding the cause and ways to prevent this problem could go on to help treat a huge range of illnesses.

**THE FUTURE**

The long-term implications of the work of the Humoral Immunity and Autoimmunity Group are vast, and could signal a dramatic change in patient care. Understanding the function and development of Tfh cells could lead to new or improved vaccines for infectious diseases, including tuberculosis, malaria and HIV, as well as explaining why the human body is unable to neutralise HIV infections with an antibody response. It is also possible that this work will go on to increase understanding of the genes and molecules that cause autoimmune disease, a goal that the team is striving to attain.

In the short term, the next step is to understand the physiological regulation of Tfh cells, with the ultimate goal of defining which factors determine their ability to create an optimal memory B cell response. The research being produced by the team suggests that Tfh development could be regulated by several other epigenetic and post-transcriptional mechanisms, and the process could be fine-tuned by microRNAs. Vinuesa and her colleagues are also currently investigating cytokines, including IFN-γ, which are secreted by haemopoietic cells. IFN-γ could be one of the contributors to the low selection threshold in germinal centres, and documenting this could provide clues about autoantibody formation and how to prevent it.

There are also metabolic factors to investigate: different forms of stress, for instance, could have an effect on the development and maintenance of follicular T cells, affecting the ratio of Tfh and Tfr cells. In parallel, the team is also taking a genetic approach to unravel the causes of autoimmunity: “Our recent work performing massively parallel sequencing of patients’ exomes promises to broaden the spectrum of monogenic or oligogenic autoimmunity and refine the diagnosis of patients with unclear diagnoses,” summarises Vinuesa. “Ultimately, this approach will pave the way to personalised medicine, and improve the lives of many suffering from these chronic debilitating conditions.”

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**INTELLIGENCE**

**SPECIALISED SUBSETS OF T FOLLICULAR HELPER CELLS IN THE CONTROL OF INFECTION AND IMMUNE PATHOLOGY**

**OBJECTIVES**

To elucidate the signals that allow B cells to produce high-affinity antibodies and become memory B cells, so that they can rapidly and effectively respond to potentially lethal infections while preventing autoimmunity.

**KEY INTERNATIONAL COLLABORATORS**

Dr Facundo Batista, London Research Institute, UK
Dr Russell Jones, McGill University, Canada
Professor Ken Smith, Dr Michelle Linterman, University of Cambridge, UK
Associate Professor Adrian Liston, University of Leuven, Belgium
Professor Pamela Schwartzberg, National Institutes of Health, USA
Associate Professor Sidonia Fagarasan, RIKEN, Japan

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**CONTACT**

Professor Carola G Vinuesa, MD, PhD
Head, Department of Pathogens and Immunity Group Leader, Humoral Immunity and Autoimmunity
Elizabeth Blackburn NHMRC Research Fellow
John Curtin School of Medical Research
Australian National University
Building 131, Garran Road, Acton
GPO Box 334 Canberra City
ACT 2601, Australia
T +61 2 6125 4500
F +61 2 6125 2595
E carola.vinuesa@anu.edu.au

**PROFESSOR CAROLA G VINUESA** was born in Spain and obtained a medical degree at the University Autonoma of Madrid. In 2000, she was awarded a PhD by the University of Birmingham. A year later, Vinuesa was the recipient of a Wellcome Trust International Travelling Fellowship to conduct postdoctoral work at the Australian National University (ANU). Since 2006, she has been leading the Humoral Immunity and Autoimmunity Group at ANU. She is currently Professor of Immunology and Head of the Pathogens and Immunity Department, and is supported by an Elizabeth Blackburn Fellowship from the NHMRC.