Role recognition

Professor John F Kearney from the University of Alabama at Birmingham in the US explains his current work investigating B cells and the increasing recognition of their significance in modern immunology

Could you begin with an overview of your professional background?

I have an unusual background, having first obtained a degree in Dentistry in Australia followed by a PhD in Immunology. I came to the University of Alabama at Birmingham (UAB) as a postdoc in the laboratories of Professors Max Cooper and Sandy Lawton, who were setting up a key research programme on the development of lymphocytes. In this role, I began to work on the development of the B cell repertoire in mouse models.

Why have B cells been understudied in comparison to T cells within the context of autoimmune and allergic phenomena, and what can be gained from increasing our understanding of the establishment of diverse B cell repertoires?

The 'hygiene hypothesis' links increasing allergic and autoimmune phenomena in humans to excessively sanitary conditions early in life, which is proposed to alter the T helper 1 (T_h1)/T_h2 balance. The T_h1/T_h2 concept was introduced about 20 years ago, and was almost universally adopted by T cell immunobiologists to explain multiple immunological phenomena to the exclusion of roles for B cells and antibodies. The revision of this hypothesis through the discovery of multiple T cell subsets and the effectiveness of drugs such as Rituximab, which depletes human B cells, have renewed interest in the role of B cells in multiple diseases.

Can you provide an insight into the different approaches you have used in the investigation of immune response to bacterial spores? What have been your most significant findings in this regard?

The bacterial spores we have studied have been from *Bacillus anthracis* and its close relatives *B. cereus* and *B. thuringiensis*. We studied these bacteria in response to a Defense Advanced Research Projects Agency (DARPA)-funded project to develop better methods for *B. anthracis* detection. From this work, we became familiarised with lung infection models, for example, which greatly helped in our investigations of the role of moulds and their conidia in lung infections and allergies.

One of the most significant, but not unexpected, findings has been the induction of serum antibodies and isolation of monoclonal antibodies by bacterial vaccination that also react with a variety of fungi and yeasts. By themselves, these organisms do not induce strong antibodies and may even suppress such antibody responses.

What have your studies revealed about the relationship between terminal deoxynucleotidyl transferase (Tdt) and B cell development?

The activity of Tdt in T and B cell development is believed to contribute to over 80 per cent of receptor diversity by adding non-templated nucleotides to gene segment joins during receptor formation. However, Tdt is not considered essential for successful gene rearrangements and its activity cannot be regulated except by knocking it out. We have shown through the overexpression of Tdt in mice that a subset of B cells that express germline encoded receptors are missing, and as a consequence important protective antibodies are absent.

Have you collaborated with other projects or laboratories over the course of your investigations? Has a multidisciplinary approach proved important to the success of the project?

I have worked with a number of colleagues, such as Professor Charles Turnbough on the *B. anthracis* work and many other individuals on B cell studies. The essence of all these collaborations has been to bring together expertise from multiple areas to build a successful combined research group. I had never seen a spore or *Aspergillus* conidia before I started.

Grant submission reviewers are often critical of investigators who do not have expertise in a part of the area she/he plans to study. I think this is very shortsighted since an outsider will bring a new outlook to a particular problem. Unfortunately, the reviewer may often be trying to preserve the status quo in a research niche.

UAB has long been a centre of excellence in immunological research. How does working in such a setting benefit your ongoing investigations?

UAB is a relatively young university and has expanded enormously since I came here. Many of us have grown up in parallel with its development, so there is a very real family spirit of camaraderie and cooperation. That is why I have stayed, and my research has benefited from long-term friendships and colleagues.

You recently received the 50th Distinguished Faculty Lecture award at your university. What was this in honour of?

This was in honour of the achievements and contributions I have made during my 40 years at UAB and the impact our studies have had on understanding of the immune system.

The nitty gritty

Scientists have long hypothesised a link between increasingly hygienic lifestyles and the rise of autoimmune disorders. A team at the **University of Alabama at Birmingham** is now looking at the role of B cells in our long-term susceptibility to diseases such as asthma and Type 1 diabetes

SINCE THE DAWN of the 20th Century, improvements in living conditions and the availability of medicines have multiplied human life expectancy. However, certain diseases have in fact increased in prevalence in recent times. Among these diseases are allergic forms of asthma and autoimmune disorders such as Type 1 diabetes, which affect individuals at ever-younger ages.

One potential explanation for these observed increases is the 'hygiene hypothesis'. Having gained currency in recent decades, the theory posits that the growth of autoimmune and allergic diseases is linked to improvements in hygiene and sanitation, as well as increases in the use of antibiotics and vaccinations. These improvements, whilst acting to prevent the spread of many diseases, have reduced or altered the exposure of young children to environmental and commensal microorganisms. As a result, the immune system of susceptible children turns its attention to normally harmless organisms or self-antigens, resulting in overzealous immune responses and culminating in allergies and autoimmune diseases which continue into adult life.

AUTOIMMUNE AND ALLERGIC DISEASE

The hygiene hypothesis provides a plausible backdrop against which experiments can be devised to investigate the induction of autoimmune and allergic diseases. Proposed mechanisms behind the theory revolve around lymphocytes, which are known to play a major role in these diseases. Lymphocytes are a type of white blood cell and come in three forms: natural killer cells, T cells and B cells.

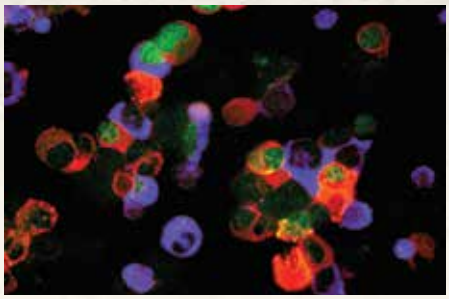
To explain the hypothesis, attention in previous research has largely focused on the role of T cells, in particular the relationship between T helper 1 (T_h1) and T_h2 subsets of CD4 T cells. From this body of work, it is thought that a lack of exposure to antigens in the first years of life results in an increase in T_h2 relative to T_h1. This leads to the production of immunoglobulin E (IgE) antibodies which may, for example, aggravate the symptoms of allergic airway associated forms of asthma.

Approaching the problem from a different angle, Professor John F Kearney and his team at the University of Alabama at Birmingham (UAB) see an increased role for B cells in such models of autoimmune disease. Whilst they believe T cells are important, they think that they are not lone actors. Kearney and colleagues hypothesise that the B cell repertoire is altered through early exposure to environmental antigens. These alterations could have long-lasting effects on adult natural antibody levels and consequently protection or susceptibility to autoimmune diseases.

FILLING THE GAS TANK

In retrospective epidemiological studies seeking to relate childhood infections with the incidence of Type 1 diabetes, a strong negative correlation has been observed with *Streptococcus pyogenes*, a bacterium which leads to common Group A streptococcal infections (GAS) such as strep throat, impetigo and scarlet fever. This correlation has been replicated in mouse models by Kearney and his team. They seek to test the hypothesis that anti-*N*-acetylglucosamine (anti-GlcNAc) antibodies generated by B lymphocytes in response to GAS or similar microorganisms protect against Type 1 diabetes. In this disease, the immune system attacks the insulin-producing beta cells located in the islets of Langerhans in the pancreas, ultimately destroying their function. After infection or immunisation with GAS, B cells produce anti-GlcNAc

Scanning electron micrograph of a cluster of *Aspergillus fumigatus* conidia with outgrowth of fungal hyphae.



Immuofluorescence photomicrograph of a smear of LPS-activated mouse B cells from a transgenic mouse expressing the short form of terminal deoxynucleotidyl transferase (sTdt). Tdt (Green), IgM (Red) and IgG (Blue). Staining shows nuclear localisation of Tdt.

antibodies, which have the potential to lessen the immune response to the GlcNAcylated molecules associated with these pancreatic beta cells.

Using this knowledge, the researchers will investigate how early immunisation influences Type 1 diabetes and asthma-like progression in mice and the mechanisms of protection against these diseases. Such work has the potential to improve diagnosis for those who are susceptible to the disease as well as open doors towards the development of prophylactic immunisations or probiotic treatment protocols.

ANTI-GlcNAc ANTIBODIES FOR ASTHMA

The UAB team has already shown the potential of anti-GlcNAc antibodies in their research on asthma models. They have found in their experiments on mice that increased exposure to antigens in the period after birth has a significant effect on antibody response in adult mice. This results in a substantial decrease in the occurrence of allergic airway-associated asthma-like disease. The antigen in question is chitin, one of the most abundant biopolymers on our planet and found in many allergen-associated organisms.

Kearney and colleagues used the fungus *Aspergillus fumigatus*, a source of chitin in its natural state, to discover whether antibodies against bacterial polysaccharides influence the interaction between *A. fumigatus*-associated allergens and receptors in the lungs. They showed that the production of anti-GlcNAc antibodies reduces the immune response to live *A. fumigatus*, proposing that these antibodies bind to the fungus, preventing it from interacting with receptors in the lung. Akin to the researchers' hypothesis regarding Type 1 diabetes, infection with GAS in early stages of life results in a reduced reaction to *A. fumigatus*. There are many other GlcNAc-containing antigens, in multiple microorganisms and parasites, which may also have the potential to increase this resistance.

DIVERSIFYING THE REPERTOIRE

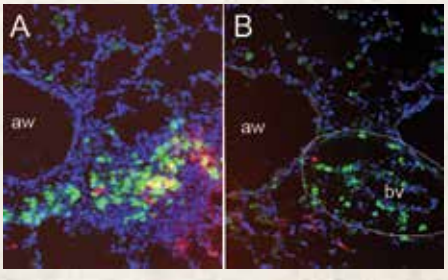
As a product of the interplay between genes and environment, no single mechanism is likely to explain the occurrence of asthmatic and allergic diseases, yet the UAB group's work highlights the importance of early exposure to antigens as per the hygiene hypothesis. Not all B cells are identical, resulting in a repertoire which can be influenced by the environment, as Kearney explains: "There is virtually nothing known about B cell repertoires in disease. All antibodies and B cells are not equal, so we need to understand how their development and function are controlled".

Rather than ignoring the importance of T_h1/T_h2 balance, this focus adds to the list of antibodies which are possible targets for disease prevention. The team's mice studies have shown that the adult B cell repertoire is heavily influenced by exposure to environmental antigens during childhood. Early exposure of mice to antigens causes changes in the B cell repertoire which result in long-term resistance to *A. fumigatus*-induced allergic airway disease. It is not only the exposure itself that matters but also its timing, as such resistance does not occur when adult mice are immunised with GAS. Certain B cell clones are thus only generated in the foetal and neonatal period and remain in the adult repertoire of antibodies.

HEALTHY STRIDES

Moving on from mouse models, Kearney and colleagues are now looking to investigate whether this cause and effect relationship can be observed in human patients. Other projects the team has been working on include looking at the function of the enzyme terminal deoxynucleotidyl transferase (TdT), whose lack of expression in the foetus contrasts with its action in B cell development in adult bone marrow.

Applying their discoveries directly to humans reinforces the potential significance of this work to public health, paving the way towards effective vaccinations against autoimmune disorders such as Type 1 diabetes and asthma.



Immuofluorescence photomicrograph of lung tissue sections stained with DAPI (blue) to detect DNA and Siglec F (green) to detect eosinophils. Mice were either treated with PBS (**A**) or heat killed Group A streptococcal (GAS) vaccine (**B**) at three days after birth.

INTELLIGENCE

THE REGULATION OF B CELL CLONAL DIVERSITY AND ITS ROLE IN DISEASE

OBJECTIVES

- To investigate the modulating role of bacteria-reactive B cells on the development and progression of allergic airway disease and Type 1 diabetes
- To test the hypothesis that alternative antibody production can influence the development of autoimmune diabetes and asthma by contributing to protection or acceleration of disease
- To assist in the development of treatments, including a vaccination approach, that will prevent or dampen the allergy-associated processes that cause asthma, and identify similar factors that influence the progression of Type 1 diabetes in susceptible individuals, which may improve diagnosis and immunisation strategies

KEY TEAM MEMBERS

Rodney King, Assistant Professor

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PROFESSOR JOHN F KEARNEY completed his PhD in Immunology at the University of Melbourne, Australia in 1973. Postdoctoral studies at the University of Alabama at Birmingham followed, before joining the microbiology staff there in 1977. The Kearney Lab has made a number of groundbreaking discoveries in fundamental cellular and molecular biology.