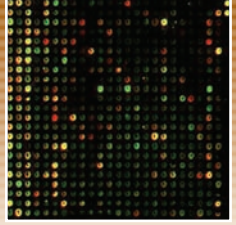
Autoimmunity Research Unit



Autoimmune Disease Mechanisms Group

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BACKGROUND

The immune system is designed to fight against pathogens, infections and also cancer cells. Another function of our immune system is to detect and eliminate potentially harmful lymphocytes, which appear during development and activation, and, for some reason, start recognising our own tissues. However, in some individuals, this safety mechanism no longer works - the harmful lymphocytes escape and then trigger autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), Inflammatory Bowel Disease (IBD), Multiple Sclerosis (MS) or Type 1 diabetes. They are the subject of several projects in the ARU.

A second theme of our research is treating the brain to cure immune disorders. Another really exciting concept emerging in our group is the connection between stress and immuno-suppression. Our collaboration with the Neuroscience Program gives us access to mice deficient in neuropeptide Y (NPY) receptors. Via activation of differing NPY receptors, NPY controls various physiological and neurological functions; appetite, bone mass, aggressive behaviors, cardiovascular function and stress. Looking at the $Y1^{-/-}$ mice, we discovered that these mice are severely immuno-suppressed suggesting a molecular model for how stress negatively effects our immune defences. We are continuing to probe other aspects of neuroimmunology.

This is a snapshot of some of the projects on offer. Depending on the student's interest we can look at other research possibilities within this framework.

PROJECT 1 — The role of B cells in autoimmunity

We have generated mice transgenic (Tg) for a cytokine named BAFF, which triggers SLE in these animals. We have discovered that the disease can occur in the absence of T-lymphocytes, which is quite surprising as SLE is supposed to be a T-cell-dependent disease. We suspect that in BAFF transgenic mice, B cells secrete some pathogenic autoantibodies, which may trigger tissue destruction in the kidney. To address this point we want to purify antibodies from the serum of sick BAFF Tg mice, inject them into normal mice and check for disease symptoms. If this works, we would like to identify the autoantigen recognized by pathogenic autoantibodies.

PROJECT 2 — The role of the neuropeptide Y1 receptor in the immune system

Y1 is expressed on many immune cells and is critical for the function of antigen presenting cells (APC) such as dendritic cells. In mice lacking Y1, APC function is impaired and T cell activation does not occur properly. Conversely, Y1 expression on T cells is key for T cell regulation. T cells lacking the Y1 receptor are hyper-responsive and can exacerbate autoimmune colitis upon transfer into lymphopenic mice. This project will focus on dissecting the signalling mechanism down-stream of Y1 in T cells and APC. Y1 can inhibit T cell activation, which suggests that Y1 signalling interferes with signalling mechanisms downstream of the T cell receptor (TCR). To help us find clues we plan to do gene profiling of WT versus $Y1^{-/-}$ T cells activated or not and identify genes that may be altered in $Y1^{-/-}$ T cells (this data will be available for further analysis to the students when they come). The plan is also to do phosphorylation assays on key components, known to be activated in response to TCR stimulation. A similar approach will be used to understand Y1 signalling in APC but this time following Toll-like receptor (TLR) activation.

PROJECT 3 — Conditional CXCR7-deficient mice

We have identified a new chemokine receptor CXCR7 which is expressed on a subset of B cells important in Autoimmunity. We have developed mice lacking CXCR7 specifically in the B cells to study the exact role of CXCR7 in B cells. We also have mice producing green fluorescent antibody forming cells and combined with B cell-specific CXCR7 knockout mice we would like to study the role of CXCR7 in the formation of B cell secreting antibodies and in immunological memory (vaccination). CXCR7 is also an angiogenic factor which promotes vessel growth in tumors. Specific deletion of CXCR7 in the vessels of mice protects these animal against tumor growth and cancer. Using *in vitro* and *in vivo* angiogenesis assay we would like to understand how CXCR7 works and from this work identify novel therapeutic targets in cancer.

Autoimmune Disease Mechanisms Selected Publications

Mackay, F.,S.A. Woodcock, P. Lawton, C. Ambrose, M.Batscher, P. Schneider. J. Tschopp, and J.L. Browning. 1999. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J. Exp. Med.* 190:1697

Mackay, F., and J. L. Browning. 2002. BAFF: a fundamental survival factor for B cells. *Nat. Rev. Immunol*. 2:465.

Sutherland, A. P., L. G. Ng, C. A. Fletcher, B. Shum, R. A. Newton, S. T. Grey, M. S. Rolph, F. Mackay, and C. R. Mackay. 2005. BAFF augments certain Th1-associated inflammatory responses. *J. Immunol.* 174:5537.

Wheway, J., C. R. Mackay, R. A. Newton, A. Sainsbury, D. Boey, H. Herzog, and F. Mackay. 2005. A fundamental bimodal role for neuropeptide Y1 receptor in the immune system. *J. Exp. Med.* 202:1527

B cell Immunobiology Group

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BACKGROUND

Antibody production is one of the immune system's major weapons in its perpetual battle against infections from invading micro-organisms. Vaccine technology has harnessed the destructive power of antibodies to provide protection against many debilitating infectious diseases. On the other hand, autoimmune diseases such as lupus, myasthenia gravis and hemolytic anemia can arise when antibodies are produced which attack the body itself. Strict controls over the immune system are essential to maintain the balance between responsiveness to structures that are 'foreign' and unresponsiveness to those that are 'self'.

The primary focus of our laboratory is B lymphocytes, the cells responsible for antibody production. We aim to identify the molecules and cells that both drive antibody production against foreign structures and prevent antibody responses against self. Our investigations utilise a sophisticated mouse model that provides the unique ability to follow B cells as they respond *in vivo* (1,2). Used in conjunction with gene targeting and complementary molecular analyses, this model allows us to identify the specific genes and signalling pathways that regulate B cell survival, proliferation, and differentiation (1-4). By understanding how B cells function we hope to reveal new strategies for improving vaccines, controlling autoimmune disease, and treating B cell malignancies.

PROJECT 1 How are memory B cells made and where do they go?

Immunity against previously encountered infections stems largely from the presence of memory cells generated during the initial immune response. Little is known about how memory B cells are produced, where they reside, and what their properties are. In this project, the production of memory B cells will be followed *in vivo* (2) and their location and gene expression determined. Techniques involved include flow cytometry, immunohistology, RT-PCR, gene array analysis, DNA sequencing, retroviral gene transduction, recombinant protein production, and Western blotting.

PROJECT 2 Switching the antibody response

Antibodies act by binding to invading structures and eliminating them. However, different types of antibodies are produced under different circumstances. Viral infections typically generate IgG antibodies, gut infections IgA, and allergens IgE. We have already developed a system for tracking the events that occur during a typical IgG antibody response (2). In this project, alternative immunisation strategies will be employed to allow identification of the molecular and cellular controls that drive B cells to switch to IgA and IgE antibody production. The results obtained will aid our understanding of mucosal and allergic immune responses.

PROJECT 3 Do TRAF2 and TRAF3 suppress lymphoma formation?

TRAF proteins act as signal transducers for members of the tumour necrosis factor receptor superfamily. We have produced mice in which expression of the TRAF2 and/or TRAF3 molecules can be inactivated in specific cell types (3). B cells that lack expression of TRAF2 or TRAF3 have a greatly extended lifespan and accumulate *in vivo*. In this project, the ability of absent TRAF2 and/or TRAF3 expression to precipitate lymphoma formation will be tested. Also, the occurrence of TRAF2 and TRAF3 mutations in naturally occurring human lymphomas will be analysed.

B cell Immunobiology Selected publications

Phan TG, Amesbury M, Gardam S, Crosbie J, Hasbold J, Hodgkin PD, Basten A, Brink R. B cell receptor-independent stimuli trigger immunoglobulin (Ig) class switch recombination and production of IgG autoantibodies by anergic self-reactive B cells. *Journal of Experimental Medicine* 2003; 197:845 860

Thien M, Phan TG, Gardam S, Amesbury M, Basten A, Mackay F, Brink R. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. *Immunity* 2004; 20(6):785-98

Grech AP, Amesbury M, Chan T, Gardam S, Basten A, Brink R. TRAF2 differentially regulates the canonical and noncanonical pathways of NF-kB activation in mature B cells. *Immunity* 2004; 21:629-642

Paus D, Phan TG, Chan TD, Gardam S, Basten A, Brink R. Antigen recognition strength regulates the choice between extrafollicular plasma cell and germinal center B cell differentiation. *Journal of Experimental Medicine* 2006; 203:1081-91

Genes and Autoimmunity Group

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BACKGROUND

Type 1 diabetes (T1D) occurs when the immune system mistakenly targets and destroys the insulin-producing beta cells within the pancreas. Beta cell specific antibodies, produced by a class of immune cells termed B lymphocytes, are highly predictive of individuals that will develop T1D both in humans and animals models of the disease such as the NOD inbred mouse strain. Although these antibodies do not seem to be directly pathogenic to beta cells, NOD mice made deficient in B lymphocytes no longer develop T1D indicating that these cells play an important role in the development of disease. Our recent research has demonstrated that B lymphocytes contribution to disease is mainly due to their unique ability to specifically take up beta cell proteins and present them in a form palatable to another class of immune cells known as T lymphocytes. After this interaction, T lymphocytes subsequently become armed to recognise and destroy the beta cells. Normally, B lymphocytes recognising the body's own proteins are eliminated in healthy mice and human individuals through a process known as tolerance. Our laboratory's main focus is to determine how tolerance mechanisms fail in T1D prone individuals, resulting in the production of B lymphocytes recognizing beta cell proteins. In addition, we aim to develop a better understanding the underlying molecular and genetic aspects leading to the development of autoreactive B lymphocytes contributing to T1D. Studying these aspects of disease may not only advance our understanding of T1D pathogenesis, but also illuminate potential new targets for early stage therapeutic intervention in humans to prevent disease.

PROJECT 1 — Pinpointing the defective tolerance mechanisms giving rise to beta cell reactive B lymphocytes in diabetes prone NOD mice and humans

B lymphocytes specific for any particular protein are normally hard to track in mice and humans due to their infrequency. To overcome this problem, we utilise genetically engineered mice where B lymphocytes are all specific for one defined protein. The target protein can be made to be exclusively expressed by beta cells, allowing us to examine the effectiveness of B lymphocyte tolerance to this type of molecule on a large scale. We have incorporated such systems into mice that are either T1D prone (i.e. NOD mice) or resistant. By comparing B lymphocyte tolerance on both of these backgrounds, we hope to gain important insights into the defective mechanisms leading to the production of beta cell specific B lymphocytes that contribute to T1D in mice. These studies will also lead to the examination of whether similar mechanisms are defective in humans developing T1D. Major techniques used in this project will include tissue culture, flow cytometry, fluorescence microscopy, and enzyme-linked immunosorbent assays.

PROJECT 2 — Genetic and molecular basis underlying the production of T1D promoting B lymphocytes

Predisposition to T1D in humans and NOD mice is controlled by many genes throughout the genome. Although the approximate location these genes are known, very few have actually been identified and little is known about how these genes lead to the development of T1D. We have recently discovered that genes in defined regions of Chromosomes 1 and 4 in NOD mice control the capacity of B lymphocytes to contribute to T1D. This project will involve performing comparisons of gene expression in different types of B lymphocytes from NOD mice and mice containing diabetes resistance variants of genes on Chromosomes 1 and 4 utilising microarray technologies. Using these studies as a starting point, we aim to identify genetic mutations leading to the production of T1D promoting B lymphocytes in NOD mice and determine whether similar genes are mutated in humans with T1D. Major techniques used in this project will include a variety of molecular biology techniques, microarrays, real-time PCR, gene sequencing, and flow cytometry.

Genes and Autoimmunity Selected Publications

Silveira PA, Johnson E, Chapman HD, Buit T, Tisch RM, and Serreze DV (2003). The Preferential Ability of B Lymphocytes to Act as Diabetogenic APC in NOD Mice Depends on Expression of Self-Antigen Specific Immunoglobulin Receptors. *European Journal of Immunology* 32: 3657-3666

Silveira PA, Dombrowski J, Johnson E, Chapman HD, Nemazee D and Serreze DV (2004). Defects in B cell selection underlie the diabetogenic antigen presenting cells in NOD mice. *Journal of Immunology* 172:5086-94

Reifsnyder PC, Li R, Silveira P, Churchill G, Serreze DV and Leiter EH (2005). Conditioning the Genome Identifies Additional Diabetes Resistance Loci in Type 1 Diabetes Resistant NOR/Lt Mice. *Genes and Immunity* 6:528-38

Silveira PA and Grey ST (2006). B cells in the spotlight: innocent bystanders or major players in the pathogenesis of Type 1 Diabetes. *Trends in Endocrinology and Metabolism*. 17:128-35