



Inflammation Group

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BACKGROUND

The immune system is responsible for defense against pathogens, but in some cases can turn against the tissues of the body and cause autoimmune disease. An understanding of the genetic and environmental components responsible for breakdown of self-tolerance and development of autoimmune disease is slowly emerging. Important tools that have contributed to this understanding are genomics techniques such as comprehensive gene microarrays, which allow assessment of gene expression in important immune cells, and new strains of knock-out, knock-in and mutant mice that are suitable for studies in disease models such as asthma, multiple sclerosis or rheumatoid arthritis.

Our laboratory is interested in two aspects of immunology: the understanding of immune and disease processes, and the development of new therapeutic approaches to inflammatory diseases. We have integrated all of the important technologies that allow us to move from gene association with a pathogenic cell type (culprit) involved with inflammation, to 'validation' of such targets using animal models or *in vitro* systems. Our laboratory also uses monoclonal antibodies (mAbs) extensively, as tools to dissect biology of important molecules. For instance mAbs can be used as reagents to assess expression of molecules by flow cytometry, assess association of molecules with disease using immunohistochemistry, and also to block the function of a molecule *in vitro* and *in vivo*, to understand the true relevance of a novel molecule.

PROJECT

1

The role of novel G-protein coupled receptors and ligands in immune cell migration and inflammation

Through mining of the immune cell transcriptome developed in our department, we identified new molecules important for subsets of dendritic cells (DCs), so called plasmacytoid DCs. We wish to understand the role of these new molecules in initiating immune responses, their role in inflammation, and how these molecules are expressed and regulated. Gene deficient knock-out mice will be used to explore the biology of these receptors. In addition, established

models of arthritis, lupus, EAE and asthma will be studied. The creation of gene-deficient mice is already underway at the company Ozgene, and represents an attractive project for a student to step into.

PROJECT

2

Use of an inflammation/suppression reporter mouse to understand critical decision points and influences on tolerance versus immune activation

We are in the process of constructing a mouse that has a red fluorescent dye attached to expression of the cytokine IL-17, and a green dye attached to expression of FoxP3. These 2 molecules are the essential elements for inflammation by Th17 T cells, or immunosuppression by T regulatory cells. The potential uses of this mouse, which is being produced by Ozgene, to dissect immune processes is unlimited. For instance, various activating stimuli can be assessed *in vivo* or *in vitro* for effects on Th17 or Treg development, and novel therapeutic agents can be tested for their effects as well. The balance between these two subsets of T cells is possibly one of the most critical factors determining immunity or autoimmunity and an understanding of all the cell types, cytokines, Toll receptors, and environmental elements that affect this balance should provide fundamental new knowledge.

Inflammation Group Selected Publications

von Andrian UH, Mackay CR. 2000. T-cell function and migration. Two sides of the same coin. *N Engl J Med* 343:1020-1034.

Jeffrey KL, Brummer T, Rolph MS, Liu SM, Callejas NA, Grumont RJ, Gillieron C, Mackay F, Grey S, Camps M, et al. 2006. Positive regulation of immune cell function and inflammatory responses by phosphatase PAC-1. *Nature Immunol* 7:274-283.

Shum B, Mackay CR, Gorgun CZ, Frost MJ, Kumar R, Hotamisligil GS, Rolph MS. 2006. The adipocyte fatty acid-binding protein aP2 is required in allergic airway inflammation. *J Clin Invest* August.

Lee H, Zahra D, Vogelzang A, Newton R, Thatcher J, Quan A, So T, Zwirner J, Koentgen F, Padjkaer SB, et al. 2006. Use of human C5aR knock-in mice to generate and assess potent anti-inflammatory monoclonal antibodies. *Nature Biotechnology* in press.



Antibody Engineering Group

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BACKGROUND

Since the advent of monoclonal antibodies in the 1970s, over twenty therapeutic antibodies have been approved by the Food and Drug Administration, with more than 150 candidates in clinical trials.

Today, monoclonal antibodies are prescribed for a wide range of disease indications including colorectal cancer (Genentech's Avastin), rheumatoid arthritis (Abbott's Humira) and asthma (Genentech's Xolair). Early monoclonals had been of murine (mouse) origin, which often limited their efficacy in humans. However, by the 1990s recombinant DNA and phage display technology had advanced to an extent that the generation of fully human monoclonal antibodies had finally become feasible. By completely bypassing the use of animals, this avoided many problems associated with earlier hybridoma technology, including immunogenicity and the loss of affinity during humanization of mouse monoclonals.

The research in our laboratory focuses on two aspects: the expansion of antibody function and the generation of antibodies against novel therapeutic targets. The expansion of antibody function involves both the generation of novel antibody formats (such as domain antibodies) as well as extending the range of environmental conditions under which antibodies can be utilized (such as high temperatures or extreme pH).

PROJECT

1

Generation of repertoires of human domain antibodies with improved intestinal availability

Most human antibodies aggregate under the highly acidic conditions encountered in the gastrointestinal tract. To overcome this limitation, our laboratory focuses on the generation of human antibody fragments with improved intestinal availability. This will enable the use of monoclonal antibodies for oral immunotherapy, opening up a new approach for the treatment of infectious and inflammatory conditions of the gastrointestinal tract. We have been able to demonstrate that domain antibodies can be selected by phage display to resist acid-induced aggregation. From this repertoire, antibodies against prototypic targets, including bacterial toxins, will be isolated by phage display. The availability of orally administrable monoclonals will allow us to generate candidates for the treatment of bacterial infections of the gastrointestinal tract and for the therapy of autoimmune diseases. This project involves a combination of techniques, including phage display, cloning, protein expression, ELISA and Biacore (SPR) analysis.

PROJECT

2

Generation of monoclonal antibodies against membrane protein targets

More than 60% of all drug targets are membrane proteins and in particular G-protein coupled receptors (GPCRs). However, membrane proteins tend to be difficult to express and only a limited number of structures and affinity reagents have been reported.

We have initiated multiple collaborations for the generation of monoclonal antibodies, both for structural studies of membrane proteins, as well as for the generation of candidate monoclonals against potential therapeutic targets. This includes CXCR7, a GPCR involved in B-cell function and angiogenesis. In addition to monoclonals, we also directly engineer the properties of membrane proteins, making them amenable to expression and structural analyses. This project involves a combination of techniques, including phage display, cloning, protein expression, ELISA and Biacore (SPR) analysis.

Antibody Engineering Group Selected Publications

Christ D, and Winter G. (2003). Identification of functional similarities between proteins using directed evolution. *Proc. Natl. Acad. Sci.*, 100:13202-13206

Christ D, Famm K, and Winter G. (2006). Tapping diversity lost in transformations - *in vitro* amplification of ligation reactions. *Nucleic Acids Res.* 34:e108

Christ D, and Winter G. (2006). Identification of protein domains by shotgun proteolysis. *J. Mol. Biol.*, 358:364-371

Christ, D., Famm, K. and Winter, G. (2007). A repertoire of aggregation-resistant human antibody domains. *Protein Engin. Des. Sel.* in press



Immunobiology Group

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BACKGROUND

The capacity of the immune system to prevent attack from infection following vaccination or an initial infection by the same pathogen lies in the ability to generate an effective primary immune response. It is also important to generate lymphocytes that will be reactivated following subsequent encounter with the same pathogen, thus providing immunological memory. This process generates populations of lymphocytes that 'remember' the initial infection and are capable of mounting a very rapid and efficient immune response.

Research performed in this laboratory is focused on understanding the regulation of the human immune system, both in normal individuals, and individuals with defined diseases, such as immunodeficiencies, that is individuals who have defects in their ability to mount a sufficient immune response, and are thus susceptible to infection with specific pathogens, such as viruses. We are particularly interested in understanding the mechanism by which the immune system responds following infections or vaccinations, thereby providing us with a 'memory' of the initial response so that following subsequent exposure to the

same infection, our immune systems will respond more rapidly.

PROJECT
1

Function of lymphocytes in female carriers of X-linked lymphoproliferative disease (XLP)

XLP is an inherited immunodeficiency passed on from mothers to sons. It is caused by mutations in a gene called SH2D1A and is characterised by increased susceptibility to infection with EBV. By studying patients with XLP, we have identified serious defects in the development, differentiation and function of their lymphocytes that most likely underlie the development of disease in these individuals. We are interested in investigating the immune systems of female carriers of XLP. This project will assess the impact of having only a single copy of the SH2D1A gene by studying the development and function of B cells, T cells and NK cells. This will be achieved by a combination of techniques including isolation of peripheral blood mononuclear cells and subsets of lymphocytes, multiparameter flow cytometry, cell sorting, *in vitro* cell culture, ELISAs, Western blotting and PCR.

PROJECT
2

Characterisation of the production of IL-21 by human lymphocytes

IL-21 was identified in 2000 as a cytokine that has pleiotropic effects on most subsets of human lymphocytes. Recently, IL-21 has been found to be overproduced in a number of animal models of human autoimmune diseases, such as mouse lupus. Our own studies have found that it is a strong activator of human B cells. Despite these findings, little is known regarding the cell types that produce IL-21, nor the signals that are required for its production and secretion. Therefore, this project will investigate the kinetics of IL-21 production by human CD4+ T cells, CD8+ T cells, NK cells and NKT cells compared to production of other cytokines that are also known to be capable of activating human B cells are over-expressed in autoimmune diseases, such as IL-6 and IL-10. This will be achieved by a combination of techniques including isolation of mononuclear cells and subsets of lymphocytes from different lymphoid tissues, multiparameter flow cytometry, cell sorting, *in vitro* cell culture, ELISAs, and PCR.

PROJECT
3

Integration of signals by human B cells stimulated by mediators of autoimmune disease

Autoimmunity can develop in humans when there is a disturbance to the production of molecules involved in

lymphocyte activation. For instance, the levels of BAFF and IL-10 are increased in the blood of some patients with diseases such as lupus and rheumatoid arthritis. Similarly, IL-21 is increased in a number of animal models of human autoimmune diseases. Molecules such as BAFF, IL-10 and IL-21 are potent activators of human B cells - thus, it is possible that aberrant production of these molecules directly contributes to the production of autoantibodies by B cells in these patients. Another stimulator of human B cells are ligands of Toll-like receptors, such as unmethylated DNA (known as CpG); these have also been implicated in autoimmune diseases. Despite these findings, the means by which the combination of these powerful stimuli induce B-cell activation remains unclear. Therefore, this project will dissect the individual and combined effects of BAFF, IL-10, CpG, and IL-21 on the activation, proliferation, survival and differentiation of human B cells. This will be achieved by a combination of techniques including isolation of mononuclear cells and subsets of lymphocytes from different lymphoid tissues, multiparameter flow cytometry, cell sorting, *in vitro* cell culture, ELISAs, and PCR.

Immunology Group Selected Publications

Avery DT, Kalled SL, Ellyard JI, Ambrose C, Bixler SA, Thien M, Brink R, Mackay F, Hodgkin PD, Tangye SG. 2003. BAFF selectively enhances the survival of plasmablasts generated from human memory B cells. *J Clin Invest* 112: 286-297

Ma CS, Hare NJ, Nichols KE, Dupre L, Andolfi G, Roncarolo MG, Adelstein S, Hodgkin PD, Tangye SG. 2005. Impaired humoral immunity in XLP is associated with defective IL-10 production by CD4+ T cells. *J Clin Invest* 115: 1049-1059.

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Vinuesa CG, Tangye SG, Moser B, Mackay CR. 2005. Follicular B helper T cells in antibody responses and autoimmunity. *Nature Rev Immunol* 5 853-865. (IF 32.7)



Autoimmunity & Gene Therapy Group

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BACKGROUND

A major problem for organisms with sophisticated immune systems is 'how can you prevent collateral damage' once the immune system is engaged, and 'how can you turn the immune system off?' Our laboratory is focused on studying these cellular pathways with an aim to understanding how they can influence the development of inflammatory diseases, such as autoimmunity and rejection of transplants. By harnessing the power of these pathways we may

eventually be able to prevent the immunological destruction of tissues and organs as it occurs during autoimmune disease and the rejection of organ transplants. A number of projects are on offer that would suit highly motivated and ambitious students.

Our other major focus is the immunology of diabetes and transplant rejection. Type 1 diabetes mellitus is an autoimmune disease caused by the specific destruction of the beta cells within the islets of Langerhans by auto-reactive lymphocytes. Currently there is no preventative cure for type 1 diabetes; however, therapies designed to eliminate the autoimmune response while preserving protective immunity would be one desirable option. The key to achieving this goal is to understand and define the 'factors' that govern how the autoimmune response is initiated and regulated.

PROJECT 1 Death of an islet

It is now recognised that apoptotic death of islet cells plays a critical role in the loss of insulin producing beta cells within the islet of Langerhans in both type 1 and type 2 diabetes. However, despite the importance of apoptosis in diabetes, little is known regarding how genes that control apoptosis are expressed in beta cells, or how these genes are regulated during the development of diabetes, or how they function when they are expressed. Accordingly, we have identified a number of genes essential for the beta cells survival/stress response, namely A20, IAP-2/BIRC-3 and ATF3. We have a number of exciting projects, which will further define the regulation and function of these genes in important animal models of disease. Techniques include analysis of gene regulation, laser capture microscopy, *in vitro* and *in vivo* analysis of beta cell function, analysis of beta cell death (e.g. signalling pathways and caspase activation), and use of animal models.

PROJECT 2 B lymphocytes and autoimmunity (type 1 diabetes)

We have identified B lymphocytes as critical players in the development of type 1 diabetes. Previous studies have shown that genetic ablation of B lymphocytes protects NOD mice (an important animal model of type 1 diabetes) from disease. Now, our recent studies have demonstrated marked anomalies in the B cell compartment of NOD mice that underscore their susceptibility to disease. By using soluble compounds to deplete B cells at any time during disease development, and through the use of newly created knockout and transgenic NOD mouse lines, we are now poised to elucidate the functional role of B

lymphocytes in disease development. Techniques involved include the use of novel animal models of autoimmunity (transgenics & knockouts), cell sorting (FACS) and lymphocyte re-constitution experiments, analysis of T and B lymphocyte function (cell signalling, antibody production, proliferation, chemotaxis, antigen presentation), phenotypic analysis (FACS), pathology (immunohistochemistry) and *in vivo* function (antibody production, T cell cytotoxic function, cell migration).

PROJECT 3 BAFF and the nature of the allo-immune response

Transplantation of islet grafts is one potential therapeutic option to restore normal blood sugar regulation in subjects with type 1 diabetes. However, the transplanted islets are destroyed by the original autoimmune response, as well as by an aggressive immune response directed against the tissue graft, referred to as the 'allo'-immune response. In studying the role of the cytokine BAFF upon T cell function, we found that it has a profound effect upon the allo-immune response, such that mice over expressing BAFF do not reject islet grafts. This is an important discovery, as it points to a previously unknown function for BAFF in regulating T cell function. The aim of this project is to understand how BAFF regulates T cell development and function. Techniques involved include *in vivo* models of T cell function, cell sorting (FACS) and lymphocyte re-constitution experiments, *in vitro* analysis of T lymphocyte function, phenotypic analysis (FACS), pathology (immunohistochemistry).

Autoimmunity and Gene Therapy Group Selected Publications

Grey, S. T., M. B. Arvelo, W. Hasenkamp, F. H. Bach, and C. Ferran. 1999. A20 inhibits cytokine-induced apoptosis and nuclear factor kappaB- dependent gene activation in islets. *J Exp Med* 190:1135.

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Hartman, M. G., D. Lu, M. L. Kim, G. J. Kociba, T. Shukri, J. Buteau, X. Wang, W. L. Frankel, D. Guttridge, M. Prentki, S. T. Grey, D. Ron, and T. Hai. 2004. Role for activating transcription factor 3 in stress-induced beta-cell apoptosis. *Mol Cell Biol* 24:5721.

NF- κ B Regulates beta cell death: a critical role for A20 in beta cell protection. D. Liuwantara, M. Elliot, M.W. Smith, A. O. Yam, S. N. Walters, E. Marino, A. McShea, S.T. Grey. *Diabetes*. 2006. In Press