# Centenary Institute

The **Key** of **Life**

## Annual Report 2002 – 2003

**Centenary Institute of Cancer Medicine and Cell Biology**

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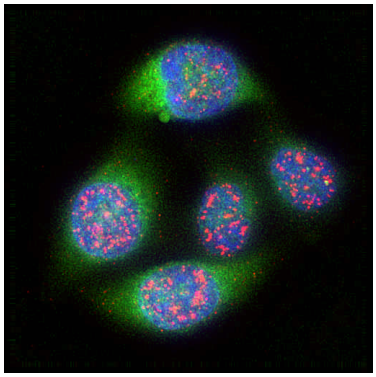
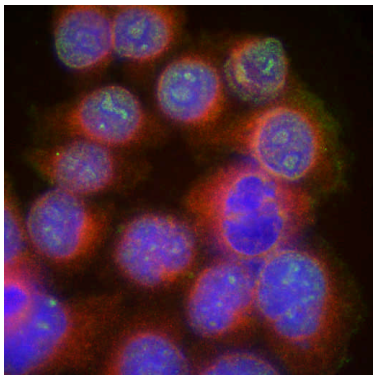
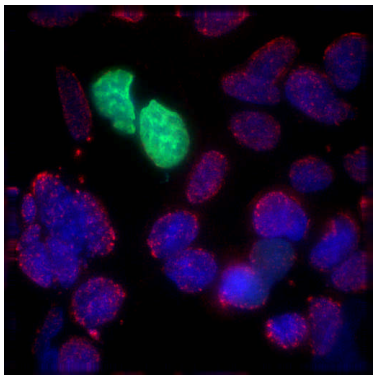
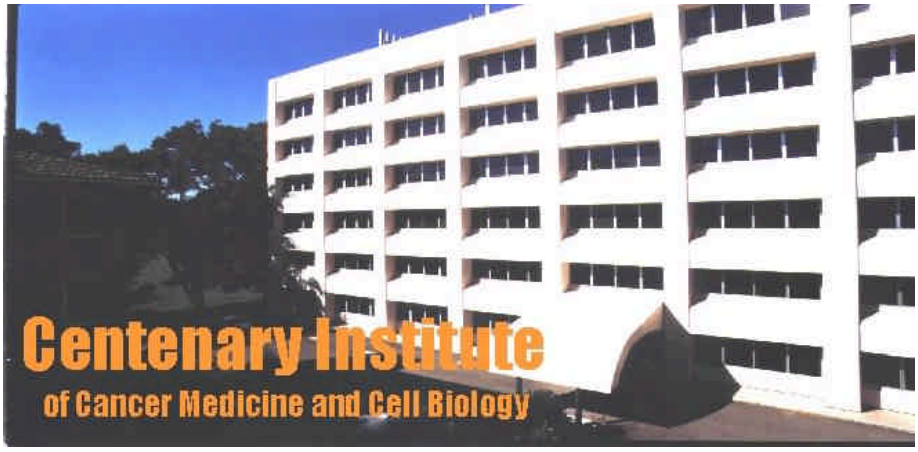






# Centenary Institute




The Centenary Institute of Cancer Medicine and Cell Biology is a centre of excellence in medical research. The Institute specialises in the diagnosis, prevention and treatment of diseases ranging from cancer, Type 1 Diabetes and allergy, to genetic heart disease and tuberculosis. Our strategy is to undertake 'cutting edge' research into the biology of normal and diseased cells, with the goal of improving our understanding of what causes disease and to translate these discoveries into clinical practice through development of new vaccines, better diagnostic tests and innovative forms of treatment.

The Centenary Institute was conceived in 1982 to commemorate the Centenaries of the University of Sydney Medical School and the Royal Prince Alfred Hospital. Formal affiliations with both organisations promote the opportunities for students to become involved in research as well as the extrapolation of research theories to practice. The Institute is located in the grounds of Royal Prince Alfred Hospital adjacent to the Medical School and University Campus, in a purpose built facility capable of accommodating a research team of up to 150 career investigators, trainees and support staff.

We have an internationally competitive multi multidisciplinary research team comprising some of the very best young scientists from major centres overseas as well as Australian researchers who have studied and/or completed fellowships on international campuses. The Institute has in excess of 680 articles published in refereed journals and books, 966 presentations at international and national conferences since 1992, and over $75 million dollars in grant funding.

# From the Director

Director Professor Tony Basten

A key to developing any long-term strategic plan is to look to a role model from whom inspiration and guidance can be sought. In my case that model has been Dr Edward Jenner[1]. In addition to his legendary discovery of smallpox vaccination, he was a truly rounded scientist who was elected to the Royal Society for his work on the habits of the cuckoo nestling and, who in his spare time, classified Sir Joseph Banks' botanical collection of Australian flora, collected during James Cook's second voyage to this land. What's more, he raised funds from the public and government to support his research and to set up vaccination clinics across the country – a true translational researcher. It is in the light of his achievements that we look at the Centenary Institute, fourteen years after its creation. Over this period we have evolved in response to the external influences exerted by a tightening fiscal climate, what I have termed the 'epidemic of Institutes'[2], and the 'biotechnology revolution', brought on by the sequencing of the human genome.

It is during times of financial constraint and changes in community perceptions of scientific research, that more than ever before we need to be perceived as having a significant contribution to make, to the world of science. A central tenet of value is international competitiveness. In order to achieve this goal the Institute has a four-pronged strategy designed: to maintain its core of expertise in Immunology; to expand its base in molecular medicine; to enhance our collaborative network; and to commercialise the Institute's research output through its biotechnology company, CenTec Limited.

Maintaining our core of expertise in immunology ensures that we can continue our high level of commitment to that discipline and its applications to infections, some forms of cancer, autoimmune disease, allergic disorders, and transplantation. By strengthening the molecular technology base in conjunction with existing expertise in immunology and cell biology, the Institute has ensured that it can be more competitive in the fields of gene therapy, carcinogenesis, genetic diseases and most recently stem cell science. Moreover, this technology base has enabled us to foster new collaborations with clinical colleagues on the local campus whose research is competitive and of mutual interest.

This network now includes interactions with the Hospital's AW Morrow Centre for Gastroenterology and Liver Disease, the Institute of Haematology, the

Sydney Cancer Centre, and the Department of Cardiology. Added to these local interactions are ongoing collaborations with over 20 national and 25 international research institutes, universities and hospitals in the USA, UK, and Europe.

The fourth prong of the Institute's research strategy has been the creation of the biotechnology company, CenTec Ltd, aimed at taking research projects at the proof of concept stage into clinical trials and ultimately the market place. CenTec is chaired by Mr MA (Tim) Besley AC and is run by the CEO Dr Paul Tan who was one of the cofounders of the well-known New Zealand biotech company, Genesis. CenTec has now been successful in obtaining two $250,000 Federal Government Biotechnology Innovation Fund grants for developmental research in the field of human monoclonal antibodies and in manufacturing a preventative vaccine for type 1 diabetes. Both grants have been topped up by a further $100,000 from the State Government.

Collectively the various prongs of our strategy are designed to position the Institute at the centre of the 'biotechnology revolution'. In other words, they are aimed at securing adequate funds for our research programme through a 50:50 balance between conventional and commercial sources, creating a niche for the Institute network in the post-genomics era of biomedical research and realising the goal of being able to vaccinate, not just against infections but against autoimmune disease, allergy, and cancer.

If we can emulate even to a modest extent, the achievements of Dr Edward Jenner, then the Institute is assured of a very bright future indeed.

[1]Friedman M, Friedland GW. Edward Jenner and vaccination. In: Medicine's 10 greatest discoveries. New Haven: Yale University Press, 1998: 65-93
[2]Basten A. The Centenary Institute of Cancer Medicine and Cell Biology. Medical Journal of Australia 1999; 171:634-7.

**Antony Basten**
**Executive Director**
Centenary Institute

# Centenary Institute Annual Research Review 2003

*'Improved quality of life for all Australians through excellence in medical research.'*

The Centenary Institute's research programme is based on a combination of cell biology, molecular medicine and immunology. There are four reasons for this: the first is the importance of studying normality in parallel with abnormality, that is, comparing healthy cells with diseased cells. Without a fundamental understanding of how normal cells work, the basis and control of cancer, as well as many other causes of death and disability will remain elusive. Disorders of cell behaviour underlie all the diseases being studied at the Institute, ranging from tumours like melanoma, infections like tuberculosis, allergic diseases like asthma, autoimmune disorders like type 1 diabetes, or cardiac diseases like hypertrophic cardiomyopathy.

Secondly, it has made good sense to capitalise on the traditional strengths of our research in immunology and its applications to cancer, infection, allergy and autoimmune diseases. Significant progress has been made within all these disease categories. Under development is a better, more effective vaccine for tuberculosis, which has emerged once again as a public health problem worldwide. A potential vaccine for insulin dependent diabetes is within two years of clinical testing and a new universal approach to the treatment of autoimmune and allergic disorders is under investigation.

## Who we are...

The third cornerstone of our research strategy has been recognition of the value of accommodating the best researchers who wish to work on the campus irrespective of their specific fields of interest. This is reflected in the recent establishment of the Ginges Molecular Cardiology Centre as well as major initiatives in gene and stem cell therapy and multi drug resistance in cancer. Given the shared technology base needed for competitive research in the post genomics era, these programmes sit comfortably alongside our long-standing immunology project portfolio.

Finally the Institute by virtue of its name also has a strong commitment to cancer research per se. In addition to the new initiative in multi drug resistance, two molecular biology teams are working on the problem of why cancer cells survive so much better than normal cells and what are the genetic defects underlying leukaemia and breast cancer with the aim of correcting these defects by gene therapy. Ultimately the focus of the cancer research programme, like those for other diseases, will be development of better prognostic tests and of preventative strategies in high risk subjects based on vaccination.

Research has been carried out by seven groups within the Institute, namely B cell Biology (the Director's Lab), T cell Biology, Autoimmunity, Gene Therapy, Mycobacterial Immunity, Liver Immunobiology and Molecular Cardiology. Some of these groups include independent laboratories with their own research focus. More detailed information on each lab's research is available in the individual laboratory research sections, from the relevant publications, or by contacting the Centenary Institute.

# The Director's Laboratory

## Head: Professor Antony Basten,
### AO FAA FTSE MBBS Dphil (Oxon) FRCP FRACP FRCPA

The research activities of the Director's Laboratory, also known as The B Cell Laboratory encompasses four different programs managed by independent scientists with the overall support and leadership of the Director.

## 1. Regulation of T Cell Dependent Antibody Responses

The first group is headed by Dr Robert Brink. This group has two major complementary areas of research:

1. The control of B lymphocyte development and antibody responses, and
2. The molecular and physiological functions of the Tumour Necrosis Factor Receptor (TNFR) family

Our major strategy in each case has been to use gene targeting in mice to establish precise experimental models with which to study physiological processes. This approach has utilised the targeted gene mutation ('gene knockout') facility which is also run within the lab. Together these investigations are identifying key molecular controls by which the immune system is regulated, particularly with regard to the production of antibodies by B cells. These findings may lead to positive interventions in the treatment of autoimmune diseases as well as improvements to current vaccine strategies.

### B Lymphocyte Responses

B lymphocytes (B cells) are continually produced in the bone marrow and can be activated by foreign antigens such as viruses and bacteria to produce antibodies which in turn eliminate that foreign antigen from the body. This process must be tightly regulated, however, in order to ensure that antibodies reactive with 'self antigens' present in cells of the host itself are not produced. To understand both the activation and inactivation of B cells more fully, we have used gene targeting to produce mice in which a significant fraction of B cells produce antibodies against the protein hen

egg lysozyme (HEL). Anti-HEL B cells in these mice can undergo all the normal events associated with B cell activation including the production of 'switched' antibodies such as IgG, IgA and IgE.

Using this model it has been shown that B cells recognising HEL as a soluble self-antigen die soon after they are produced and are thus prevented from making antibodies in response to antigen. Nevertheless, two stimuli associated with the onset of autoimmune disease, bacterial LPS and the cytokine BAFF, were found to trigger the production of switched and unswitched antibodies in this model. Further study of this system will allow us to identify the mechanisms whereby these agents precipitate autoimmune disease. Analysis of responses to HEL as a model foreign antigen have also begun which are aimed at devising a system for generating rapid and potent IgG antibody responses of potential value to vaccine technology.

### TNF Receptor Superfamily

The receptors of the TNFR superfamily play critical roles in regulation of the immune system. CD40 and the receptors for BAFF are constitutively present on B cells, whereas T cells express a wide range of TNFR family members following activation by antigen. TNFRs regulate lymphocyte development and responses by delivering intracellular signals with the capacity to alter cellular survival, proliferation, and differentiation. Cytoplasmic proteins known as TRAFs play an important role in the transmission of these signals. In our laboratory we are currently characterising the role of the TRAF2 protein in immune responses.

A constitutive lack TRAF2 expression is embryo lethal. Mice have therefore been produced in which the TRAF2 gene is active but flanked in critical regions by loxP recombination sites. In such mice, the TRAF2 gene can be specifically inactivated by expression of the Cre DNA recombinase. These mice have revealed TRAF2 to be an important regulator of B cell development due to its role in BAFF signalling. TRAF2 deficient B cells have greatly improved survival indicating that mutations in TRAF2 may be associated with autoantibody production and B cell lymphoma. Preliminary results also indicate a critical regulatory role for TRAF2 in T cell responses. Investigations of T cell responses are being pursued in collaboration with Dr Barbara Fazekas de St Groth, head of the T Cell Biology Group.

### Targeted Gene Mutation Facility

The facility has now produced over a dozen genetically modified mouse lines using gene targeting in embryonic stem (ES) cells. These have all been produced on the inbred C57BL/6 background, a property essential for investigation of the immune system. In addition to conventional 'gene knockouts', the facility has developed state-of-the art gene modification technology that allows introduction of specific mutations into genes including loxP sites to facilitate conditional gene knockouts in combination with Cre transgenic lines. A number of new lines are currently under production for the B Cell Biology laboratory as well as other laboratories within the Institute.

## 2. Human Immune Responses in Health and Disease

The ability 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 leads to the appearance of populations of memory lymphocytes which 'remember' the initial infection and are capable of mounting much more rapid and efficient immune responses than naïve cells. To unravel the complexities of human lymphocyte differentiation, Dr Stuart Tangye and his group are examining human T and B-lymphocytes in health and disease.

## Normal B cell Differentiation

His group has been able to demonstrate that human naïve and memory B cells are both capable of becoming immunoglobulin (Ig)-secreting cells (ISC) and that these processes require the cells to undergo three or more cell divisions. A striking difference is that memory B cells undergo cell division and differentiation into effector B cells, at a much greater rate than do naïve B cells.
Furthermore, the effector cells generated from activated memory B cells acquire characteristics which provides them with growth and survival advantages over memory B cells that remain undifferentiated. These intrinsic differences between naïve and memory B cells provide a mechanism for the accelerated immune response following secondary challenge with an infectious pathogen. Exploiting such differences may contribute to the development of improved vaccines, as well as enhancing the immune system of immunodeficient individuals. He and his colleagues have also identified a population of plasma cells in the human spleen that is likely to make important contributions to long term humoral immunity.

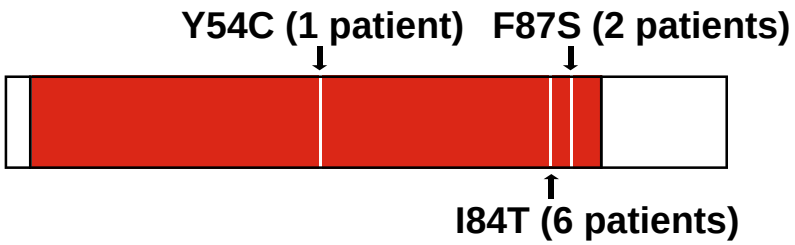
## Normal T cell Differentiation

More recently he has begun to investigate the requirements for human naïve T cells to become cytokine-producing effector cells. Using cells isolated from umbilical cord blood and adult peripheral blood, it has been found that T cells produce cytokines associated with a Th1-type of response (interleukin [IL]-2, IFN-g, TNF-a) independent of cell division while the expression of Th2-type cytokines (IL-4, IL-10) required four or more cell divisions.

## X-linked Lymphoproliferative Syndrome (XLP)

Patients with XLP have difficulty in responding to Epstein-Barr virus (EBV), the virus responsible for causing glandular fever/infectious mononucleosis. In these individuals, infection with EBV is often fatal – those that survive often develop other complications, such as hypogammaglobulinaemia or B cell lymphoma. This disease is caused by mutations in a gene (SH2D1A) that encodes a protein (SAP) involved in transmitting signals from the outside of lymphocytes to the nucleus of the cells. SAP achieves this by binding to certain receptors expressed on the surface of immune cells. When delivered normally, these signals efficiently guide the appropriate response of the immune cells. However, in patients diagnosed with XLP, immune cells respond inefficiently and as a result an often fatal immunodeficiency develops. Understanding the intracellular signals that are affected in this disease will reveal the function that they have in normal immune cells and how they may be corrected in XLP.

To date nine patients from three different families affected by XLP have been identified and the mutation in their SAP gene responsible for the disease characterised (Figure below). The gene mutations identified so far greatly reduce the amount of SAP protein in the cells. Several aspects of the signal transduction pathways utilised by receptors that are expressed on the surface of lymphocytes and associate with SAP are currently being characterised and further investigated. For instance, the mechanism whereby SAP can bind to one cell surface receptor (CD84) has been established and the consequences of mutations in SAP on this process determined. The ultimate aim is to understand how a single gene mutation can manifest as an immunodeficiency syndrome.



## 3. The Molecular Basis of Antibody Maturation

This program is led by Dr Chris Jolly. The major soluble agents providing a barrier to infections are antibodies, otherwise known as Immunoglobulins or Ig. Immunoglobulins of enormous diversity are continuously produced by B lineage cells (B cells). In response to infection, B cell clones secreting Ig capable of binding to the invading organism, or to it's products, are activated and compete with each other to select those clones producing Ig antibody of greatest affinity for the target antigen. Selection is associated with the diversification of the initially 'naïve' IgM class of antibodies into 'memory' IgG, A and E classes ('Ig class switching'), and with the introduction of mutations into the Ig genes ('Ig somatic mutation'), class switching allows the secreted antibodies to deal optimally with different types of infections in different tissues. At the same time somatic mutations modify the binding characteristics of the affected Igs, thereby increasing the efficiency of selection for optimal antibodies and fill 'holes' in the antibody repertoire. The program has two goals:

a. Understanding of the mechanisms of Ig somatic mutation and class switching,
b. Use that understanding to develop a novel in vitro technology for identifying, optimising and producing human antibodies for clinical use.

If proof of concept for b. is confirmed, this platform technology has enormous potential for application to infections, cancer and allergies, and in generating income for our future research.

Diversification of the antibody repertoire occurs in two stages: primarily by rearrangement ('V(D)J recombination') of Ig V, D and J gene segments in precursor B cells resident in the bone marrow, and then by somatic mutation and class switch recombination of these rearranged Ig genes when mature B cells are activated. Immunoglobulin somatic mutation and class switching are central to the maturation of the immune response and the generation of a 'memory' response. Furthermore, dysregulation of these processes probably contributes to some human leukaemias.

Over the last year we have examined the role of the DNA repair protein, DNA-PKcs, in switching. DNA-PKcs is an extremely large protein (470 kDa) that, in association with the KU80 and KU70 proteins, forms a complex which binds to broken DNA ends, phosphorylates itself and other proteins (hence DNA-Dependent Protein Kinase), and thus mediates Non-Homologous End Joining (NHEJ) of the broken DNA. It was already known that deficiency of KU80 or KU70 blocks both V(D)J recombination and class switching. This proved that NHEJ is required for these processes. Deficiency for DNA-PKcs also blocks V(D) J recombination, but its role in class switching was unknown. To define the role of DNA-PKcs in switching, the B cell compartment in SCID mice was rescued using V(D)J-rearranged Ig transgenes. SCID mice carry a point mutation in the DNA-PKcs gene that completely removes DNA-dependent protein kinase activity and reduces DNA-PKcs protein levels by about 90%. We found that switching to all isotypes was reduced by about 60% in Ig-transgenic SCID mice compared to control mice carrying the same Ig transgenes on a $RAG^{-/-}$ background. This contrasts with V(D)J coding joint formation, which is almost completely blocked by the SCID mutation, and with V(D)J signal joint formation, which is blocked by about 90%. At the same time another group who were examining switching in DNA-$PKcs^{-/-}$ mice found that switching to all isotypes (other than IgGI) was completely blocked in the absence of any DNA-PKcs protein. Thus, switching differs from V(D)J recombination: DNA-PKcs clearly has a role, but its kinase activity appears to be dispensable. In other words the DNA repair pathway involved in switching is subtly different from known mammalian DNA repair pathways.

## 4. Molecular and Cellular Studies in Multiple Myeloma

This form of chronic leukaemia remains one of the most refractory leukaemias to treat. Consequently more information is needed about the molecular basis of myeloma and new approaches to therapy are required. These are the target of research by Dr's Joy Ho and Daniel Sze who are working in the Centenary Institute under a collaborative agreement with Royal Prince Alfred Hospital's Institute of Haematology.

Dr Ho and her group have demonstrated the presence of chromosomal translocations involving the immunoglobulin genes in bone marrow of myeloma patients. They have then set up assays for the different translocation partners by using fluorescent in situ hybridisation (FISH) and real-time PCR to analyse their impact on disease behaviour. This has enabled them to play a major role in the current national myeloma trial (MM6, Australian Leukaemia and Lymphoma Study Group), designed to investigate the potential of thalidomide, an inhibitor of the mitogenic growth factor FGF, in myeloma patients undergoing stem cell transplantation. They are studying the impact of the t(4;14) translocation and the expression of FGFR3 [(one of the candidate oncogenes of t(4:14)] on the efficacy of thalidomide. A second project aims to identify and characterise precursor cells of myeloma. Potential precursor cells are purified from the peripheral blood and their clonality established by sequencing of the immunoglobulin variable region, and the presence of switch translocations is being studied and compared between the malignant cells and the cells from which they may have developed. This will enable the group to define when the switch translocations arose in the development of the plasma cell, and whether they play a role in oncogenesis.

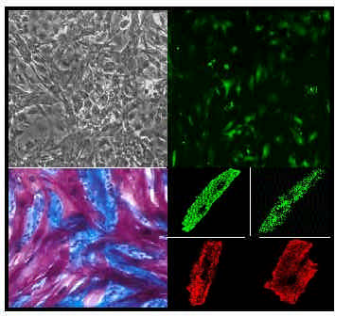
Dr Sze has identified $CD8^+$ T cell expansions in patients with multiple myeloma which appear to be associated with a good prognosis. To identify the specificity of these T cell clones, he has successfully transferred the specific T cell receptor gene segments (TCRV$\alpha$18 and V$\beta$1) of one patient, as well as the CD8 costimulatory molecule into a human T cell line that itself does not express T cell receptor. The same approach is now being applied to another four patients. The next target is to prepare cDNA libraries and screen for the associated T cell specific peptide antigens.

Since myeloma idiotype is a potential antigen being recognised by the T cell receptor, he is also testing for anti-tumour idiotype responses. To that end he has performed a pilot study using web-based bioinformatics to detect immunodominant peptides (ie peptides with a greater chance of eliciting an immune response). Results from the web-based immunodominant peptide idiotype specific predictions will allow him to correlate them with the results obtained for the antigen specificity of the T cell receptor (see above).

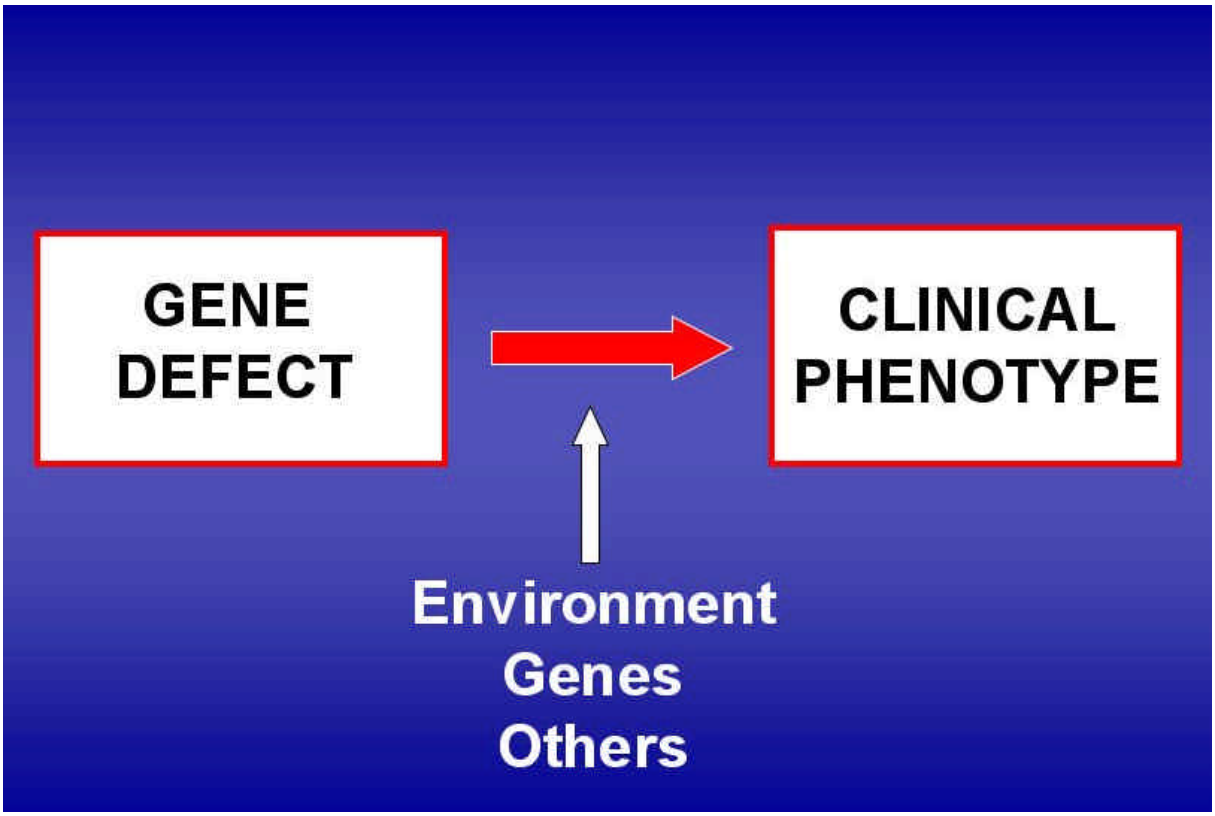
# Molecular Cardiology Laboratory

## Head: Dr Christopher Semsarian, MB BS PhD FRACP

The Agnes Ginges Centre for Molecular Cardiology was established in January 2002. The main focus of this new laboratory is the study of heart muscle disorders, and in particular those diseases which are inherited and therefore caused by DNA abnormalities called mutations. An important example is the disorder, *hypertrophic cardiomyopathy* (HCM), which was the first cardiovascular disease in which a genetic basis was identified and has therefore acted as a model for inherited heart muscle disorders. The disease is characterised by marked thickening of the heart muscle and occurs in approximately 1 in 500 people. It can result in a variety of symptoms including chest pain, shortness of breath, and syncope (fainting), and can lead to both heart failure and sudden death, often during sporting activities. HCM is the commonest cause of sudden death in the young.



*The aims of this Laboratory are to understand how these mutations lead to disease and in particular, identifying which individuals are at highest risk of dying suddenly. These aims are being addressed in three concurrent sets of studies involving isolated cells, genetically-modified mice, and humans with inherited cardiovascular disorders attending the Genetic Heart Disease Clinic at Royal Prince Alfred Hospital, Sydney. In addition to understanding how these gene defects cause disease, factors which may either worsen or improve the clinical outcome are being studied. These include other genes and environmental factors such as exercise and diet, and the use of novel drug treatments in order to improve the outcome of this disease.*




*The following two projects currently being undertaken illustrate the types of research which are being performed in the Laboratory:*

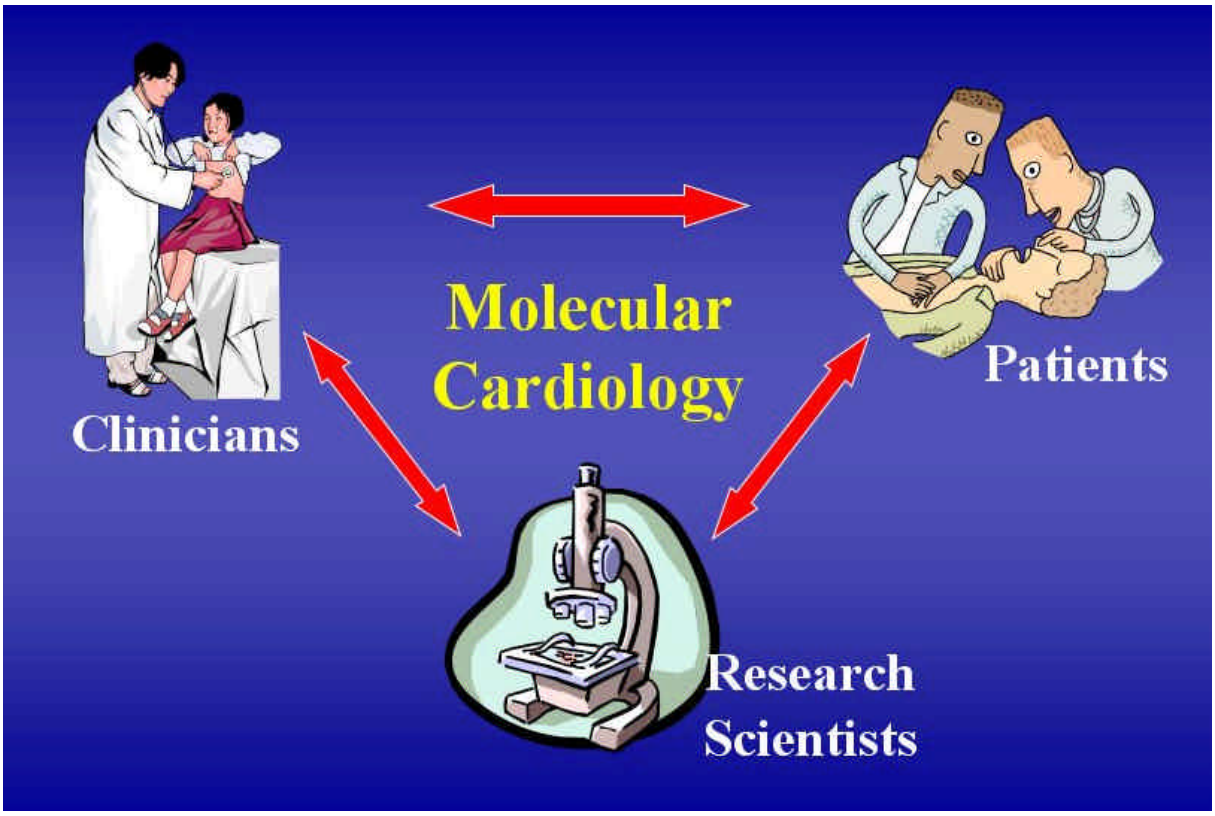
### Mutation Screening in HCM Families

Over 250 families with HCM from around Australia currently attend the Clinic, and are currently being screened for gene defects known to cause HCM. risk stratification strategies to determine which individuals are most likely to develop sudden death are `being investigated, thereby allowing those individuals to benefit from preventative therapies.

### Mouse Models of Heart Disease

Genetically engineered mouse models of HCM have been developed to allow the study of disease pathogenesis in genetic heart disorders, and in particular, to evaluate the role various environmental influences such as exercise. To this end, a “mouse gym” has been developed with allows the study of the effect of different types of exercise, e.g. running and swimming, on the progression of heart disease and its important complications such as heart failure and sudden death. High-tech facilities have also been developed to allow very accurate analysis of the mouse heart, e.g. mouse blood pressure, ECGs, and cardiac ultrasound.

Understanding the basic biology of heart muscle function and therefore defining ways to treat heart muscle disorders clearly has wider implications for a variety of cardiovascular disorders. Specifically, patients with heart muscle disease ranging from cardiomyopathies to coronary artery disease will be expected to gain most from identification of new therapeutic strategies developed as a result of a better understanding of the molecular biology of heart muscle. The potential therapeutic boundaries are limitless. Proper integration of molecular biology, genetic technologies and clinical medicine will ultimately reduce human diseases and prolong life. It is the focus of the Agnes Ginges Centre for Molecular Cardiology to realise these goals.




The Agnes Ginges Centre for Molecular Cardiology acknowledges support and donations from the National Health and Medical Research Council, the National Heart Foundation of Australia, the Rebecca L. Cooper Foundation, the Department of Cardiology at Royal Prince Alfred Hospital and the ongoing support of Mr and Mrs Berel Ginges.

# Immune Regulation Laboratory

## Head: Dr. Stuart Tangye, BSc PhD

The ability 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. We are interested in unravelling the complexities of human lymphocyte differentiation. This is being achieved by examining human T and B-lymphocytes. Specifically, research performed by Dr Tangye's group is aimed at investigating

(a) intrinsic differences in the biological behaviour between naïve B cells (that have not seen antigen) and memory B cells (those that have previously responded to antigen);
(b) the signals required to generate effector B cells from populations of naïve and memory B cells;
(c) the development of memory B cells in vivo, in particular in situations where individuals exhibit impaired immune responses, such as patients suffering from immunodeficiencies, and the ability to correct cellular defects in these immunodeficient states;
(d) the biology of effector B cells generated during an immune response in vivo; and
(e) the regulation of cytokine production by neonatal and adult T cells.

All of these studies utilise the division-tracking dye CFSE, which permits determination of the number of times that a lymphocyte has divided. It is also possible to examine the changes that occur within lymphocytes at different division numbers.

Another area of interest being pursued by Dr Tangye is the inherited immunodeficiency X-linked lymphoproliferative disease. The majority of patients with XLP have difficulty in responding to Epstein-Barr virus (EBV), the virus responsible for causing glandular fever/infectious mononucleosis. In these individuals, infection with EBV is often fatal those that survive often develop other complications, such as hypogammaglobulinaemia or B-cell lymphoma.

This disease is caused by mutations in a gene (SH2D1A) that encodes a protein (SAP) involved in transmitting signals from the outside of lymphocytes to the nucleus of the cells. SAP achieves this by binding to certain receptors expressed on the surface of immune cells. When delivered normally, these signals efficiently guide the appropriate response of the immune cells. However, in patients diagnosed with XLP, immune cells respond inefficiently and, as a result, an often-fatal immunodeficiency develops. Understanding the intracellular signals that are affected in this disease will reveal the function that they have in normal immune cells and how they may be corrected in XLP.

# RESULTS

### B-cell Differentiation

Currently, we have been able to demonstrate that human naïve and memory B cells are both capable of becoming immunoglobulin (Ig)-secreting cells (ISC) and that these processes require the cells to undergo three or more cell divisions. A striking difference is that memory B cells undergo cell division and differentiation into effector B cells, at a much greater rate than do naïve B cells. Furthermore, the effector cells generated from activated memory B cells acquire characteristics which provides them with growth and survival advantages over memory B cells that remain undifferentiated. These intrinsic differences between naïve and memory B cells provide a mechanism for the accelerated immune response following secondary challenge with an infectious pathogen. Exploiting such differences may contribute to the development of improved vaccines, as well as enhancing the immune system of immunodeficient individuals. We have also identified a population of plasma cells in the human spleen that is likely to make important contributions to long-term humoral immunity.

### T-cell Differentiation

We have also begun to investigate the requirements for human naïve T cells to become cytokine-producing effector cells. Using cells isolated from umbilical cord blood and adult peripheral blood, we have found that T cells produce cytokines associated with a Th1-type of response (interleukin [IL]-2, IFN-? , TNF-?) independent of cell division while the expression of Th2-type cytokines (IL-4, IL-10) required four or more cell divisions.

### XLP

We have identified 8 patients from 3 different families affected by XLP, and characterised the mutation in their SAP gene that is responsible for this disease (Figure below). The gene mutations identified so far greatly reduce the amount of SAP protein in the cells. Several aspects of the signal transduction pathways utilised by receptors that are expressed on the surface of lymphocytes and associate with SAP are currently being characterised and further investigated. For instance, we have established the mechanism by which SAP can bind to one cell surface receptor (CD84) and have determined the consequences of mutations in SAP on this process. We are also extending our studies on lymphocyte differentiation by examining the behaviour of T and B cells from XLP patients for their ability to undergo differentiation in vitro in an attempt to understand how a single gene mutation can manifest as an immunodeficiency syndrome.

# Cancer Drug Resistance Group
# Special Projects

### Head: Dr. John Allen, BSc PhD

## The problem

Almost a third of us will experience cancer sometime in our lives. The cure rates vary greatly for different types of cancer and the chances are much better if it is caught early. Overall, about half of all cancer patients are cured, mostly by a combination of surgery and radiotherapy. However, in cases where cancer is disseminated throughout the body, as in leukaemia, or metastatic cancer, chemotherapy is the main treatment option. In such cases the long term survival rate is much poorer - around 1 in 5. An over-riding reason for this poor result is drug resistance. Cancers that are initially sensitive to the drugs used to treat grow back after remission, in a drug resistant form. Remarkably, such acquired drug resistance frequently manifests as multidrug resistance, whereby the tumours become refractory to many drugs, and not only those they have been treated with. This greatly restricts the possibilities for alternative treatment.

## Goals

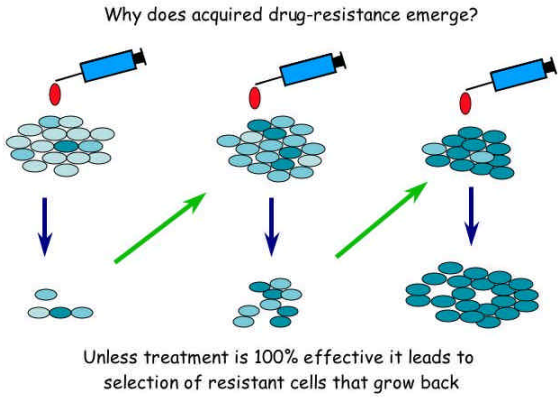
The Cancer Drug Resistance group was established in 2002 in order to:

- identify mechanisms of resistance to anticancer drugs,
- evaluate their clinical significance and
- identify ways of overcoming or avoiding drug resistance.

A gratifying aspect of work in this field is that the results of basic research can be tried quickly in clinical trials, and we are in an excellent position to exploit advances in basic research through a close relationship with the Sydney Cancer Centre.

## Research

The group presently focuses on resistance to new anticancer drugs and agents that modify drug resistance. The work is highly varied - we employ molecular, cellular and physiological techniques. Several research projects are presently underway.




### 1. Interactions of new anticancer drugs with multidrug transporter proteins.

We have a three-year grant from the NHMRC (2003-5) to determine whether one prominent source of drug resistance - multidrug transporter proteins - affects the efficacy and/or pharmacology of new anticancer drugs on trial at the Sydney Cancer Centre (Glivec, Iressa, Flavopiridol, NV-06, Epothilone B and others). Some of these (e.g. Glivec and Iressa) are targetted to genetic defects that underlie the development of specific cancers. Such "magic bullets" have low toxicity compared to traditional anticancer drugs and so may be taken daily over long periods of time, converting cancer from an acute, lethal disease to a chronic manageable one. However, under this type of treatment regimen, the opportunity for ultimate emergence of drug resistance is greatly enhanced. Kara Tanabe is systematically evaluating the interactions of the new drugs with seven known multidrug transporter proteins. We are collaborating in this work with the Sydney Cancer Centre and the Childrens' Cancer Institute Australia.

### 2. Role of transport in the dose-limiting toxicity of Irinotecan and its amelioration.

Irinotecan is a relatively new drug active against several types of cancer. Like most anticancer drugs, it has severe side effects (principally diarrhoea) that limit the dose that can be administered. Peter Tobin, a PhD student based at the Sydney University School of Pharmacology, is investigating the causes of those side effects in our lab, and several alternative ways of ameliorating them that are undergoing clinical trials at the Sydney Cancer Centre. Peter has shown that at least one of the compounds that relieve or prevent the dose-limiting toxicity (DLT) of irinotecan affects the activity of drug transporter proteins *in vitro*. Analogous effects in patients may alter the transport of irinotecan and it's toxic metabolites into the intestine, a question that is presently under investigation in a physiological model. An issue of concern is whether such compounds could inadvertently alter the patients' exposure to the drug.

## 3. Functional consequences of ethnic polymorphisms in the ABCG2 transporter

There are sometimes large differences between ethnic groups in their responses to certain drugs - a problem of particular significance in a country as ethnically diverse as Australia. A likely reason for such differences is inheritance of different versions of particular genes, called "single nucleotide polymorphisms" (SNPs). Our Honours student, Tracy Murray, is determining whether specific SNPs in the gene for the multidrug transporter protein ABCG2 may be responsible for differences in the response of Caucasian, Asian and Indian populations to the anticancer drug Irinotecan. By introducing polymorphic versions of the transporter into model cell lines, the effects of the SNPs on the transporter's behaviour can be evaluated. Two properties are of particular interest. One is the rate at which irinotecan and it's metabolites are transported across epithelial membranes, which could affect its bodily uptake, distribution and elimination. The other is the transporter's ability to expel irinotecan and other anticancer drugs from cancer cells, which is a source of resistance to the drugs. This work is a collaboration with the National Cancer Centre in Singapore.

## 4. Contribution of defective apoptosis pathways to the drug resistance of melanoma.

Australia has the world's highest incidence of melanoma. Although it is cured effectively by early surgery, it is one of the cancers most resistant to chemotherapy. Left too long untreated, melanoma is almost invariably fatal. In the coming year we will begin investigation of drug resistance in melanoma, focusing on the failure of melanoma cells to "commit suicide" (undergo apoptosis) when damaged by anticancer drugs. This work will be pursued by combining a mouse melanoma model with genetically manipulated mouse lines that have specific defects in their apoptosis pathways. The work is in collaboration with the Walter and Eliza Hall Institute in Melbourne and the Netherlands Cancer Institute in Amsterdam.

# Liver Immunobiology Laboratory

## HEAD: Professor Geoffrey McCaughan, MBBS(Hons) FRCAP PhD

Diseases of the liver are common in our society. Disorders of the liver include viral hepatitis, cirrhosis and liver cancer. The incidence of liver disease has increased by about 300% in the last 20 years such that now approximately 1% of the Australian community is infected with the hepatitis C virus (HCV) while up to 20% may be affected by fatty liver.

Cirrhosis - Cirrhosis of the liver is a condition in which the normal cellular composition and architecture of the liver is severely altered. Abnormal structures, particularly collagen fibres, many activated star-shaped (stellate) cells and excessive numbers of activated T cells are present.

Differential expression of genes in cirrhosis - Gene expression in various types of chronic disease, including human hepatitis C infection, is being assessed using DNA arrays. DNA array is a new technology in which the level of expression activity of thousands of genes is assessed. This approach identified some disease processes and individual genes not previously known to be involved in the development of cirrhosis. Individual genes identified this way and now studied in more detail include RERE, DDR1, PLGF and FIt-1. These genes are involved in the key processes of cell death, tissue remodelling and production of new blood vessels.

Hepatitis B and C virus infections - The long term outcomes of HCV infection post transplant are currently being analysed on an Australia wide basis. This study has found that worse outcomes are associated with recipient HCV genotype 4 and use of older and fatty donor livers.

Alcoholic Liver Disease - A joint project of the RPAH Drug and Alcohol Department and the AW Morrow Gastroenterology and Liver Centre is investigating the progressive pathogenesis of alcoholic liver disease in humans using DNA microarray, RNA quantitation and localisation of proteins in liver cells. In this way we have found that a group of proteins called annexins are interesting.

Peptidases of the DP-IV family in liver disease - The DPIV gene family consists of dipeptidyl peptidase IV (DPIV/CD26), fibroblast activation protein (FAP), DP8, DP9, DP-Like-1 (DPL-1) and DPL-2. The DNA sequences encoding DP8, DP9 and DPL-2 were first cloned here in the Liver

Centre at RPAH. In particular we are interested in understanding the roles of these enzymes in the pathogenesis of cirrhosis. DPIV has roles in the endocrine and immune systems, digestion, kidney function, tumour growth and cirrhosis. We have found that the amount of FAP in liver correlates with the severity of fibrosis and that cells that make FAP in liver lie alongside collagen fibres (Fig. 1). We anticipate that, like DPIV and FAP, DP8 and DP9 have roles in lymphocyte proliferation, cancer and/or cirrhosis. In addition, we have found that inhibitors of DPIV that were previously used by others to identify roles of DPIV also inhibit DP8 and DP9. Therefore, roles of DPIV identified using inhibitors could as well or instead be roles of DP8 and/or DP9. Analyses of these enzymes are improving our understanding of the structure and functions of the whole DPIV gene family.

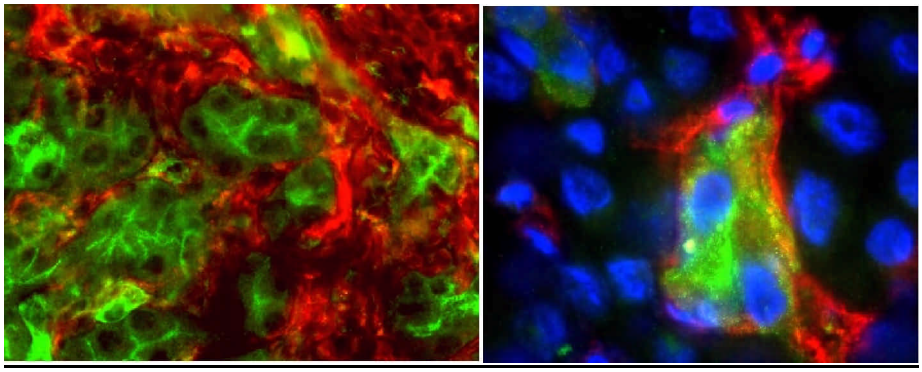


Fig. 1 Cells that make FAP (red) also make collagen fibres (green), which are the major component of the scars that form in chronically damaged liver.

## Liver Immunobiology

The broad objective of this project is to understand how a subset of white blood cells (CD8 T cells) of the immune system interacts for the first time with molecules expressed within the liver and the consequences of this primary interaction. Recognition of antigen by naïve CD8 T cells is known to occur only within lymphoid tissues (LN and spleen) and leads to T cell activation, proliferation and generation of cytotoxic T cells (CTL) able to kill target cells expressing the specific antigen. Results from the Liver Immunobiology group in the AW Morrow Gastroenterology and Liver Centre at RPAH, suggest that the liver is an exception to this general rule as primary T cell activation can occur within the liver as well. However, in contrast to T cell activation within lymphoid tissues, primary T cell activation within the liver leads to uneffective CTL and premature T cell death. This model may explain why liver grafts are easily accepted in transplantation. Furthermore, it is possible that liver-specific viruses such as hepatitis C virus may exploit this liver tolerogenic property to persist in the host.

To investigate the recirculation and fate of CD8 T cells, the main T cell subset involved in transplant rejection and viral clearance, the Liver Immunobiology group has developed three different animal models of immune-mediated hepatitis using genetically modified (transgenic) mice. In these models, the fate of autoreactive T cells specific for liver antigens is followed by injecting T cells specific for a particular antigen into transgenic mice expressing the relevant antigen at different sites within the animal.

- In one of these models, in which antigen expression is restricted to hepatocytes and lymph node dendritic cells, this group has shown that in addition to the lymph nodes, naïve CD8 T cells circulating in the blood may be directly activated in the liver by hepatocytes. Current data suggests that the liver may compete with within lymphoid tissues for primary T cell activation and may interfere with the outcome of the immune response. These results have great relevance to liver transplantation and hepatic autoimmune diseases, since T cell activation by liver cells may lead to tolerance and explain some of the tolerogenic properties of the liver.

- In a second model, despite antigen being expressed in every tissue, the liver of recipient mice was the only solid organ in which antigen-specific T cells were retained and activated. In this model, activated T cells induced an acute but transient hepatitis. It was shown that hepatocytes were killed indirectly by cytopathic cytokines such as TNF? and IFN? rather than direct CTL killing. This was the first study describing the mechanisms of “bystander hepatitis”.

- In both models described above, hepatitis is a transient phenomenon. After the onset of hepatitis, activated T cells die and hepatitis is resolved. Experiments performed by the Liver Immunobiology group suggest that T cell death may result from the lack of costimulatory molecules on hepatocytes that are essential to induce the long-term survival of activated T cells. This group has therefore generated transgenic mice expressing such a costimulatory molecule on hepatocytes. Preliminary results suggest that costimulatory molecule expression on hepatocytes increases the survival of antigen-specific CD8 T cells. Surviving T cells keep infiltrating the liver in a similar manner as T cells infiltrating the liver of patients with a mild form of hepatitis. Work is underway to induce an acute form of the disease in these mice. This transgenic model provides the first animal model of chronic immune mediated hepatitis.

This research would provide new strategies to increase the survival of organ transplants and new possibilities for treatment of autoimmune disease.

## Liver Transplantation

Rejection of transplanted organs.
Rejection is the major complication of liver transplantation. It is due to the immune system of the recipient, which recognises the transplant as “foreign” and attacks it, just as it would an infection. Immunosuppressive drugs are given to transplant patients to control rejection, however these can have severe side effects and need to be used for life. A further problem is that such drugs are not effective in controlling a form of rejection called chronic rejection, which produces a gradual decrease in function of the transplanted organ, often occurring months to years after transplantation.

Liver transplants are not rejected in some animals.
In animal models, transplanted livers are often not rejected, even though the recipient receives no immunosuppressive drugs. It is important to find out why this happens to understand how we might prevent rejection of transplants of livers and other organs in humans. Researchers in the AW Morrow Gastroenterology and Liver Centre at RPAH have been examining this model and were the first to observe that acceptance of the liver is due to white blood cells from the donor that are carried in the transplanted liver. If these white blood cells are removed, the liver is rejected, while injecting white blood cells from the donor into the recipient restores liver acceptance.

The paradox of liver transplant acceptance.
The examination of the immune mechanism of liver transplant acceptance showed an unexpected finding. There was more rapid and extensive immune activation in the recipients that accepted their livers than in the rejecting recipients, however, this activation appeared to exhaust itself. As immunosuppressive drugs inhibit immune activation it is possible that these drugs, used to control rejection, also inhibit tolerance. This was indeed the case and one drug, methylprednisolone, was found to inhibit liver transplant tolerance in the animal model.

These findings have led to several areas of investigation including using donor white blood cells to promote acceptance of transplanted organs, screening commonly-used immunosuppressive drugs for their effects on liver transplant tolerance, using early immune activation as a diagnostic marker for human liver transplant acceptance and further studies of the mechanism of donor leucocyte-induced liver acceptance. Figure X below shows the proposed mechanism that leads to acceptance of liver transplants by abnormal activation and death of the recipient T cells that would otherwise reject the transplant.

Donor white cells induce transplant acceptance - Research at the Centenary Institute was the first to show that injecting these cells at the time of transplantation could result in long-term acceptance of transplanted kidneys in an animal model whereas they reject in about a week if left untreated.

Effects of immunosuppressive drugs on liver transplants - The finding at the Centenary Institute that there was rapid immune activation during acceptance of liver or kidney transplants led to screening of immunosuppressive drugs for their effects on liver acceptance. The effects are being examined in a collaboration between the A.W. Morrow Gastroenterology and Liver Centre and the Department of Surgery at the University of Western Australia. Monitoring of liver transplant acceptance - The finding of rapid immune activation early during liver acceptance in the animal model has led to a collaboration between the AW Morrow Gastroenterology and Liver Centre and the Departments of Surgery and Gastroenterology at Princess Alexandra Hospital, Brisbane. This is investigating the early changes in liver transplant patients to establish whether early immune activation might be a useful diagnostic marker to identify those patients who will not have major problems with rejection and so might benefit from reduction or elimination of their immunosuppressive drugs.

What is the mechanism of acceptance? - Researchers at the AW Morrow Gastroenterology and Liver Centre have been at the forefront of development for methods to examine the basic molecular processes of liver transplant acceptance. These include quantitative real-time polymerase chain reaction analysis for measuring very low levels of gene expression in tissues and also gene microarray analysis, where the expression of thousands of genes can be simultaneously measured.

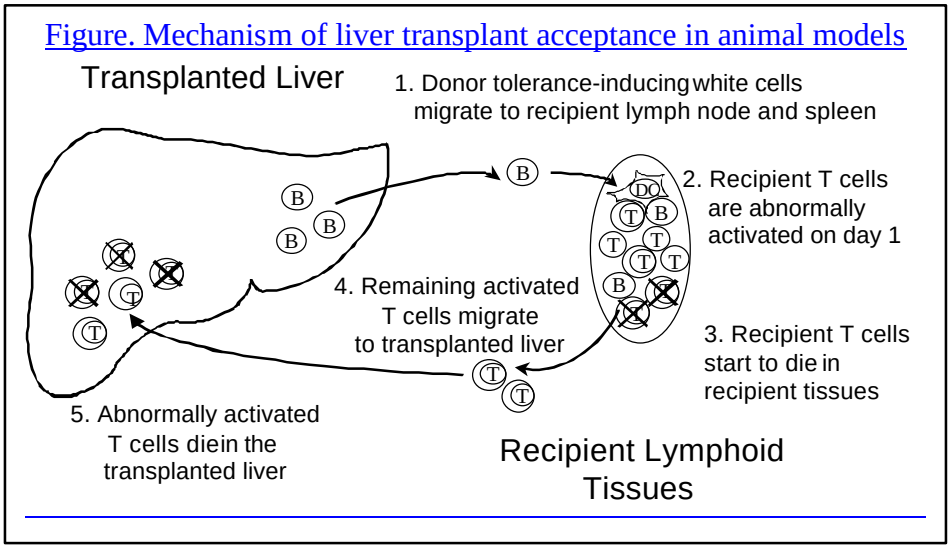



Figure X. Mechanism of liver transplant acceptance in animal models

## Xenotransplantation

A potential means of alleviating the current critical shortage of organs for transplant is through the use of other species as donors (xenotransplantation). For a variety of ethical and practical reasons, the pig is the most likely candidate donor species. However, xenotransplantation has several technical barriers. A significant barrier known as delayed xenograft rejection is a response mediated by the cells of the recipient's immune system, most notably natural killer (NK) cells and macrophages. Researchers in the AW Morrow Gastroenterology and Liver Centre at RPAH are developing strategies to overcome this barrier.

Protection of pig cells from human NK cell-mediated damage by the expression of HLA class I molecules – Human NK cells are inhibited from killing target cells that express human leucocyte antigen (HLA) class I molecules. We have shown that genetic manipulation of the pig cells so that they express these human molecules protects them from NK cell-mediated damage. We have also demonstrated that these human molecules can be genetically modified so that they have the additional property of not eliciting attack by recipient T cells. Such mutant molecules have potential for a therapeutic role in xenotransplantation.

Detecting and Characterising porcine molecules which activate human NK cells –Recent data from our group suggests that pig cells express molecules that can bind to the human NK cell activating receptor NKG2D. Such an interaction can directly activate NK cells. Thus, these porcine molecules are likely targets for therapeutic intervention. We are currently identifying and characterising these molecules.

# Mycobacterial Research Laboratory


## HEAD : Professor Warwick Britton,

**PhD, MBBS(hons) BSc (med) FRACP FRCP FRCPA DTM&H**


Mycobacteria are major human pathogens with *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis (TB), responsible for over three million deaths per year. Resistance to mycobacterial infection is dependent upon the generation of a sustained immune response and requires both T cell activation and the generation of an inflammatory response to contain the infection within a granuloma. The current vaccine against TB, known as BCG, is not effective long term thus a new and more protective vaccine is urgently needed. Research within the mycobacterial group is focused on both the generation and testing of new vaccines and understanding the cellular immune response, particularly how granulomas form and are maintained.

## Vaccination against tuberculosis.

The group is undertaking a number of complementary approaches to develop a new vaccine to combat the continuing problem of TB. In recent years the lab has focussed on the use of DNA vaccines that make important mycobacterial antigens, and recent results have shown that the addition of cytokines during DNA immunisation can improve the effectiveness of these vaccines. DNA and protein vaccines are also being developed that 'target' antigen presenting cells; these cells are needed to initiate immune responses required for optimal protection against TB. Dr Triccas and his team have extended the vaccine work by developing and assessing live vaccines as new anti-TB candidates. One approach under investigation is to use as vaccines attenuated, or 'handicapped' forms of *M. tuberculosis*. These vaccine candidates are very similar to the bacterium that causes TB but have been altered such that they do not cause disease. The aim of these studies is to determine if these live vaccines can stimulate the right type of immune response needed to fight TB and prevent infection. Preliminary results undertaken by Dr Triccas and Rachel Pinto, in collaboration with the laboratory of Professor Brigitte Gicquel at the Pasteur Institute in Paris, France, have shown that in our mouse model of TB some of these attenuated strains were significantly better than the BCG vaccine at restricting *M. tuberculosis* growth. We are encouraged by these results and we are now determining if this new vaccine shows an adequate safety profile for use in human populations.

In a differing approach Dr Triccas and colleagues are attempting to improve the current BCG vaccine by developing forms of the vaccine that make important mycobacterial antigens, either those lacking from BCG or those demonstrated to be necessary for a protective immune response. The addition of *M. tuberculosis*-specific antigens to BCG may allow the generation of a recombinant vaccine that is antigenically similar to *M. tuberculosis*, but lacking the pathogenicity of the virulent bacteria. We are also engineering BCG to produce mammalian molecules important in the host immune response, such as cytokines and chemokines, as these BCG strains may serve to enhance the effectiveness of BCG-based vaccines and therapeutic agents.

## Mycobacterial virulence and gene expression.

Dr Triccas and colleagues are also investigating the role of certain components of *M. tuberculosis* in the ability of the bacterium to cause disease. This is being done by analysing the expression during infection of a subset of mycobacterial genes, and these studies have been facilitated by the development of a form of the reporter protein GFP that permits more accurate assessment of changes in mycobacterial gene expression. The team is constructing forms of the tuberculosis bacterium that lack these '*in vivo*-expressed' genes, to determine the direct role of these components in disease caused by the bacterium.

Apart from investigating changes in bacterial gene expression during infection, the group is also investigating the effect of *M. tuberculosis* infection on the response of the host. This work, performed by Gabriella Scandurra and Dr Rohan Williams, uses DNA microarrays to investigate the change in expression of 1000's of macrophage genes after infection with *M. tuberculosis*. Initial results have shown that the macrophage, which is the major host cell for *M. tuberculosis* within the immune system, alters expression of many of its genes to respond to infection. Some of the important genes influenced by *M. tuberculosis* infection are now being studied in more detail to determine if they can exploited as components of new strategies to control infection with virulent mycobacteria.

## Macrophage activation and containment of mycobacterial infection.

Mycobacteria enter the body and are taken up by macrophages which, upon activation by T cells, kill the bacilli. TB is uniquely adapted to resist macrophage killing and if they avoid initial destruction bacilli can reside, often for decades, in a latent state in the lung contained within a small granuloma. Granulomas form when cells, predominantly macrophages and T cells, migrate from the blood into the infected tissue and surround infected macrophages to prevent further bacterial spread (Figure 1). Work in the lab has demonstrated a role for the cytokine TNF in regulating chemokine levels (chemical messengers that facilitate cell migration), and the chemokines influenced are essential for cell recruitment, granuloma formation and clearance of mycobacterial infection. This work was the topic of the doctoral studies of Dr Daniel Roach, whose PhD thesis was conferred in 2002. Over the past year Dr Saunders' research has extended these studies and shown that both macrophage and T cell derived TNF are involved in controlling the migration of T cells to the site of infection. Further we have recently shown for the first time, that both soluble and membrane bound TNF are required for sustained immunity to *M. tuberculosis.*

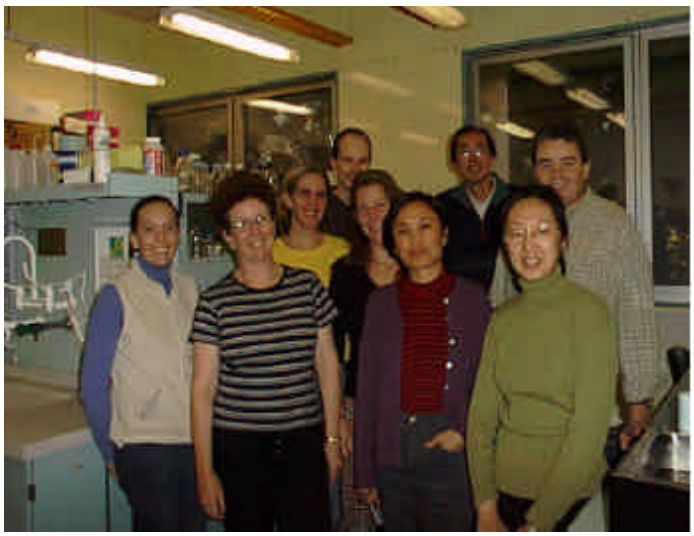
## Genetic susceptibility to *M. tuberculosis.*

Over 2 billion people are infected with tuberculosis but most never develop clinical disease, however around 10 million people a year do go on to develop tuberculosis disease. It is not well understood why some infected individuals develop clinical disease yet others remain healthy, but many cases are due to reactivation of dormant organisms within the macrophage. We know that declining socio-economic conditions, HIV co-infection, and some genetic risk factors such as HLA type contribute to the likelihood of an individual developing disease, but current known factors are insufficient to fully account for the risk of an infected individual developing disease.

Dr Saunders and Dr Fernando are investigating another potential risk factor involved in the development of tuberculosis, namely P2X7 receptor function. A natural compound, ATP, when added to the macrophage is able to kill tuberculosis organisms residing within the macrophage. This process occurs when ATP activates the P2X7 receptor leading to mycobacterial killing. We have recently identified a mutation in the P2X7 receptor and have shown that individuals who have this mutation are unable to respond to ATP and are unable to kill tuberculosis. We have now commenced a larger study to the frequency of this mutation in TB patients, healthy household contacts and the general population to determine if this mutation in the P2X7 receptor is a risk factor for the development of tuberculosis disease.

# T Cell Biology Group

## HEAD: A/Professor Barbara Fazekas de St Groth, BSc MBBS PhD



The T cell Biology group has two main areas of research. The first relates to questions of basic immunology, such as how dendritic cells activate T cells to different programs of differentiation, and how T cells compete with each other for access to antigen and other factors. Our second area of interest is the environmental and immunological factors responsible for susceptibility to autoimmune and allergic diseases. Such diseases include juvenile diabetes, MS and rheumatoid arthritis, inflammatory bowel disease, and asthma. Recent work within the group has highlighted the importance of particular subset of T lymphocytes in preventing such diseases, providing new lines of inquiry into their cause. These “regulatory” T lymphocytes have potent ability suppress the activity of dendritic cells, the cells that first stimulate T lymphocytes to make an immune response. When too few of these regulatory T lymphocytes are active, dendritic cells can cause T lymphocytes to respond to substances that would normally be ignored, such as normal bowel bacteria, in the case of inflammatory bowel disease, and inhaled house dust mite particles, in the case of asthma.

One unique insight that has come out of our research is that a link exists between the activity of regulatory T lymphocytes and environmental exposure to micro-organisms. It has been known for some time that a lack of exposure to micro-organisms can predispose to development of asthma and other immunological diseases. This link, known as the 'hygiene hypothesis', has often been explained by the ability of certain infectious diseases to skew the immune response away from allergic responses. While this hypothesis can explain the link between hygiene and asthma, it predicts that hygiene should protect against immune diseases of the opposite type, such as diabetes and inflammatory bowel disease. The opposite is actually the case - diabetes and inflammatory bowel disease are more common under hygienic conditions. By making a detailed study of how regulatory T lymphocytes function in our animal models, we have found evidence that the link between hygiene and immunological disease is actually a link between hygiene and deficient regulatory T cell function. Thus by understanding exactly how regulatory T lymphocytes function, and how their function is influenced by environmental exposure to micro-organisms, we will be able to devise ways to overcome the harmful effects of the hygienic Western lifestyle on the immune system.

One spin-off from our basic research has been a clinical trial of immunological adjuvant therapy in ovarian cancer. The immune response to cancer is often too little, too late. Normal regulatory T lymphocyte function may be contributing to the problem by suppressing the response to cancers. When regulatory T cell numbers are too low, it may be possible to stimulate unusual immune responses that will help to fight cancer. We have used this insight to design a trial of immuno-adjuvant therapy in ovarian cancer patients who have been given chemotherapy and are deficient in regulatory T cells as a consequence. The trial is being conducted in association with the Sydney Cancer Centre.

### Effect of CD4+ T cell precursor frequency and antigen dose on the generation of memory cells.

Our published data regarding competition between T cells of the same specificity suggests that the effect of initial precursor frequency should lessen during the course of an immune response. To test this, a range of doses of transgenic T cells (covering a thousandfold range) were transferred into syngeneic hosts prior to immunisation with a constant dose of antigen. By day 7, the mean difference between cell numbers in the groups that received the highest and lowest cell doses had narrowed to twelvefold, and by day 42, the difference was only threefold. These data indicate that the effect of precursor frequency on the size of the memory pool is far less than previously estimated. In addition, because the recipients of the lowest cell doses had precursor frequencies in the physiological range ($10^{-5}$ to $10^{-6}$), the data indicate that competition for antigen is not a response to abnormally high precursor frequencies, but is a stable feature of the T cell response at all precursor frequencies.

### High affinity competition for antigen reduces proliferation and cytokine production of lower affinity $CD4^+$ T cells.

We have established an *in vivo* model of T cell competition to investigate the role of competition in T cell affinity maturation. The model makes use of 3 strains of MHC Class-II restricted T cell receptor transgenic mice of varying specificity and affinity. By labelling a cohort of cells with CFSE, we are able to track proliferation of antigen reactive T cells and detect changes in the response due to competition by T cells of a different affinity and/or specificity. In the presence of a higher affinity response to the same antigen, the number of cells recruited into division is unaffected, but proliferation and cytokine production by lower affinity T cells is reduced during the later stages of the response. Competition by cells of different antigen specificity has no effect on the total number of dividing cells, but reduces production of cytokines such as IFN-? at the later stages of the response. The data suggest that competition for antigen-MHC complexes increases over the course of an immune response, and profoundly affects acquisition of effector function by dividing T cells. Antigen non-specific competition, mediated at the level of molecular interactions other than that of TCR with specific peptide/MHC, can also diminish acquisition of effector function as the immune response progresses.

### Phenotype of self-specific T cells undergoing tolerance induction in the periphery.

A number of mechanisms are believed to be involved in the induction of self tolerance in the T cell compartment. Although the primary site of T cell tolerance induction is the thymus, peripheral mechanisms are required to deal with cells specific for antigens that are under represented in the thymus. By studying the responses of cells expressing a T cell receptor specific for moth cytochrome *c*, and cross-reactive with the allo-MHC molecule $IA^s$ and with several endogenous mls

antigens, we have been able to compare the process of tolerance induction to different types of self antigen with that of cells undergoing deletion in response to intravenous peptide. Deletion involved a rapid and self-limited burst of proliferation, whereas proliferation during tolerogenic responses to moth cytochrome *c* expressed as a neo-self antigen, and to mls, was more prolonged and resembled that seen in the immunogenic response to subcutaneous peptide/CFA. Duration of IL-2 receptor expression was consistent with the proliferation pattern. In both types of responses, the majority of divided cells disappeared within days to weeks of the response, so that cell numbers were not a reliable guide to the eventual phenotype of the response.

## A T cell receptor transgenic model of transplant rejection and tolerance.

Numerous techniques to induce transplant tolerance have been demonstrated in rodents, but have not proven successful when attempted in clinical trials. This lack of applicability to humans has been attributed to major differences in immune systems. However a second possible explanation is a lack of understanding of the precise immunological mechanisms involved in graft rejection and tolerance. In order to apply T cell receptor transgenic technology to the study of graft rejection, we have set up a model in which CD4+ T cells specific for moth cytochrome *c*, and cross-reactive with the allo-MHC molecule IAs, are present as a tracer population within the mice rejecting IAs-bearing skin grafts. Control syngeneic grafts have been performed for comparison, with tempo for complete rejection defined in each group. We have used daily histological examination and immunohistochemical staining to track the progression of the antigen specific T cell response. The early appearance of an allospecific T cell infiltrate will subsequently be investigated to determine cellular phenotype, with tolerogenic manoeuvres applied to assess their effect on the allo-immune response. Additionally, models using only class I or class II allo-MHC differences will be used to determine their relative contribution to the balance between transplant tolerance or rejection.

## T cell-dendritic cell regulatory networks in lymphopaenic mice prone to developing colitis.

Some inbred mouse strains in our colony spontaneously develop a form of inflammatory bowel disease resembling human ulcerative colitis. The disease occurs only in mouse strains that are relatively lymphopaenic, and correlates with infection *Helicobacter bilis*, although other organisms may also be involved. Development of colitis in lymphopaenic mice is associated with the presence of hyperstimulatory dendritic cells, which express higher levels of the costimulatory molecules CD80 and CD86, as well as a number of members of the TNF superfamily. We suggest that the disease process in lymphopaenic mice results from a breakdown of the regulatory circuit that normally exists between T cells and dendritic cells, resulting in antigen specific activation and proliferation of naïve low affinity T cells responding to gut flora and possibly autoantigens. To further test the involvement of the CD28-CD80/86 costimulatory pathway on low affinity T cell activation to endogenous antigens, spontaneous proliferation of donor cells from CD28 gene knockout mice was compared with that of cells from C57BL/6 controls after transfer into syngeneic C57BL/6 Rag-1$^{-/-}$ recipients. Spontaneous proliferation of the CD28-deficient cells was almost completely abrogated, indicating the importance of CD28-CD80/86 costimulation in driving spontaneous proliferation, the prelude to development of colitis. We are investigating the regulatory network responsible for holding CD80/86 expression in check, in the hope of gaining further insight into the aetiology of colitis and other autoimmune diseases.

## Regulatory T cell function in genetically manipulated mice prone to inflammatory disease.

Several strains of genetically manipulated mice develop spontaneous inflammation restricted to the gastrointestinal tract. These include mice deficient in IL-10 and IL-2. In addition, some strains of genetically manipulated mice that develop inflammatory lymphocytosis, such as those deficient in CTLA-4 and TGF-beta, are also susceptible to bowel inflammation. Because independent studies have implicated these four molecules in the function of regulatory T cells, we have been investigating the link between defective regulatory T cell function and inflammatory bowel disease in the 4 genetically manipulated mouse strains.

IL-2 is critical in maintaining expression of the IL-2 receptor alpha chain CD25 in the periphery. Hence IL-2-deficient animals do not have detectable CD25-expressing CD4 T cells in the periphery. Nonetheless, the mice are not as prone to develop inflammatory lymphocytosis as CTLA-4 and TGF-beta knockout animals, suggesting that they retain some regulatory T cell function. In addition, they suffer from a concurrent proliferative defect due to IL-2 deficiency. When mixed bone marrow chimeras between IL-2 deficient and sufficient bone marrow are made, differentiation of IL-2 deficient cells into either primed (CD44 high, CD25 negative) or regulatory (CD44 intermediate to high, CD25 positive) T cells is still partially suppressed, indicating that IL-2 secreted by neighbouring cells cannot completely compensate for an intrinsic lack of IL-2 production by each individual T cell. These data indicate that regulatory T cells make IL-2 in vivo, and that they are highly reliant on IL-2 for survival.

## Regulatory T cells in patients with inflammatory bowel disease.

In this study we are comparing the number and phenotype of CD4+CD25+ regulatory T cells in peripheral blood, colon draining lymph nodes and colonic mucosa of patients with inflammatory bowel disease (either Crohn's disease or ulcerative colitis) and controls who have undergone bowel surgery for other conditions. The unequivocal identification of regulatory T cells in humans is made more difficult by the large number of primed cells expressing low to medium levels of CD25. This population overlies a substantial part of the regulatory T cell gate. A number of other gating strategies have been attempted, using a panel of cell surface markers including CD45RA/RO, CD44, CD152, GITR, CD95, CD27, MHC class II and CD134. So far, the most stable phenotypic difference between activated/memory and regulatory T cells is a slight reduction in CD4 expression in regulatory T cells. This reduction is also seen in murine regulatory T cells, suggesting that it represents a conserved characteristic of regulatory T cells.

## Reconstitution of dendritic cell subpopulations in vivo.

As part of a study of the functions of lymphoid and myeloid dendritic cells in vivo, we have been attempting to restrict IE expression to lymphoid dendritic cells, so that the response of IE-restricted T cell receptor transgenic T cells can be used as a readout of dendritic cell function. One promising method to achieve this end is to create chimaeras in an IE tolerant host that does not express peripheral IE, using bone marrow or thymic dendritic cell precursors from an IE-expressing donor. We have previously made use of irradiation (4-500 Rads) to "condition" the host for reconstitution by bone marrow and thymic precursors. However the sensitivity of lymphocytes to irradiation caused even lightly irradiated mice to become lymphopaenic, disturbing the function of T cells

administered weeks after the irradiation. The use of a conditioning regime that spared lymphocytes was therefore tested. Mice were pretreated with 5-fluoro-uracil, which differentially targets dividing cells without affecting resting lymphocytes. This protocol resulted in excellent reconstitution of dendritic cells from bone marrow precursors.

## A pilot study of dendritic cell immunotherapy in ovarian cancer.

We are conducting a pilot study of dendritic cell immunotherapy for stage III ovarian cancer. The treatment makes use of autologous tumour RNA prepared from tissue obtained during surgery. Three intranodal injections of 5-10x$10^6$ monocyte-derived, cytokine-matured RNA-pulsed DCs are administered after successful completion of 6 courses of chemotherapy. Of 33 patients who provided tumour samples in the past 2 years, only 14 have proven suitable for the trial. The others were either found not to have stage III disease at surgery, or did not respond adequately to chemotherapy prior to immunotherapy. We have now treated 8 patients with DCs. Of these, 6 experienced recurrent disease within 2-5 months of immunotherapy (ie within 9-12 months of diagnosis). These patients were those with bulky disease at diagnosis or partial rather than complete remission in response to chemotherapy. The two patients without recurrence are currently 13 and 15 months post-diagnosis. Both had less bulky disease and a full response to chemotherapy. These results suggest that DC immunotherapy is likely to be of little benefit to patients with significant residual disease at the time of treatment.

# Gene Therapy Research

## HEAD: A/Professor John Rasko, BSc (Med) MBBS PhD FRCPA FRACP

The Gene Therapy Research Group was established in 1999 as the first laboratory of the Integrated Cancer Programme through joint funding from the Sydney Cancer Centre and Centenary Institute. In early 2002 the lab settled in to newly-appointed laboratory space on the second floor of the Institute. The broad aim of the Gene Therapy Research Group is to overcome the barriers to successful human gene therapy. The main areas of study include: 1) viral vectors and their cognate receptors; 2) gene silencing and mechanisms of gene expression control; and 3) models of systemic and stem cell gene delivery. The following highlights for 2002 represent some of our ongoing projects.

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*We are grateful for funding from the NHMRC, ARC, Leukaemia Foundation of NSW, Rebecca L Cooper Foundation, and the Ernest Heine Family Foundation*

## Transcription Factors

To explore mechanisms of gene expression we have detailed the molecular and cellular biology of important transcription factors. A unique transcription factor, CTCF, has been shown by our collaborators at the National Institutes of Health and Fred Hutchinson Cancer Research Center in the USA to act as a tumour suppressor gene, genetic insulator, and determinant of imprinting (in which inheritance occurs from one parent only). Based on our report in Cancer Research in 2001, the highly conserved zinc-finger transcription factor CTCF is now known to be of central importance in the regulation of proliferation-controlling genes. Our paper also demonstrated for the first time that normal CTCF negatively regulates cell proliferation through unique effects on all phases of the cell cycle. In developing this theme of molecular controls of cellular processes, we published a paper in the *Proceedings of the National Academy of Science, USA*, detailing the cloning and functional analysis of a novel related protein which we called BORIS (for Brother of the Regulator of Imprinted Sites). BORIS is present only in the testis, and expressed in a mutually exclusive manner with CTCF during male germ cell development. Our working hypotheses for BORIS and CTCF acting successively to govern epigenetic states in normal development, and for their rivalry caused by aberrant expression of BORIS in cancer is currently being tested. The cloning and initial characterization of mouse and human BORIS genes provide new opportunities for understanding the molecular mechanics of epigenetic reprogramming, both in normal development and in tumorigenesis.

## Hartnup disorder

Hartnup disorder is an inborn error of renal and gastrointestinal neutral amino acid transport. The cloning and functional characterisation of the 'system $B^{0,}$' neutral amino acid transporter SLC1A5 led to it being proposed as a candidate gene for Hartnup disorder which we explored as a means to understand the molecular pathogenesis of this interesting disease. Linkage analysis performed at 19q13.3, the chromosomal position of *SLC1A5*, was suggestive of an association with the Hartnup phenotype in some families. However, *SLC1A5* was not linked to the Hartnup phenotype in other families. Linkage analysis also excluded an alternative candidate region at 11q13 implicated by a putative mouse model for Hartnup disorder. Sequencing of the coding region of *SLC1A5* in Hartnup patients revealed two coding region polymorphisms. These mutations did not alter the predicted amino acid sequence of *SLC1A5* and were considered unlikely to play a role in Hartnup disorder. There were no mutations in splice sites flanking each exon. Quantitative RT-PCR of *SLC1A5* messenger RNA in affected and unaffected subjects did not support systemic differences in expression as an explanation for Hartnup disorder. The analysis of these six unrelated Hartnup pedigrees studied was published in the *Journal of Inherited Metabolic Disease*. Examination of linkage at 19q13.3, polymorphisms in the coding sequence and quantitation of expression of *SLC1A5* did not suffice to explain the defect in neutral amino acid transport. In continuing to make use of the precious material supplied by members of the Hartnup families, we have commenced a genome-wide search for other candidate genes.

## Gene Therapy

The safe introduction of healthy genes into patients with genetic disorders could effectively cure inherited genetic disorders such as some cancers, haemophilia, and immunodeficiency disorders as well as infectious diseases such as HIV. The early success of gene transfer into mice has not been reproduced in dogs, monkeys, baboons or humans. Success in transferring genes into relevant animal models will represent a large step towards successful gene therapy in humans. The overall focus of this study continues to be to improve gene delivery to the precursor cells of all blood cells, known as haemopoietic stem cells. Haemopoietic stem cells have the capacity to

divide to produce billions of progeny cells throughout a lifetime and it is these progeny that form the basis of our immune system. We and colleagues at the Fred Hutchinson Cancer Research Center in Seattle have built on observations concerning gene transfer into haemopoietic stem cells made in a paper published in 2001 in the journal Blood. In that paper, the effects of using a unique retroviral coat protein to enhance gene delivery were described. In an effort to improve hematopoietic stem cell gene transfer rates using gibbon ape leukemia virus (GALV)-pseudotype retroviral vectors in baboons, the same collaboration examined preselection of transduced green fluorescent protein (GFP)-expressing CD34-enriched marrow cells. After transduction on fibronectin, cells were cultured for an additional 2 days to allow for expression of GFP. GFP-expressing cells were enriched by fluorescence-activated cell sorting and infused together with cells from the unselected fractions after myeloablative irradiation of the recipient. Three other animals were transplanted with GFP-transduced CD34-enriched cells without prior GFP selection (GU). At 4 weeks after transplant, the percentage of GFP-expressing white blood cells was significantly higher in the GS group (6.6%) than in the GU group (1.3%) ( $p < 0.002$). The higher gene transfer levels in the animals transplanted with GS cells gradually declined, and by day 100 after transplant, gene transfer levels were similar in both groups. PCR analysis performed on genomic DNA isolated from peripheral blood cells demonstrated that the decline in GFP-positive cells was due to the loss of gene-marked cells and not due to loss of expression. These results published in *Human Gene Therapy* show that transplantation of CD34-positive marrow cells selected for GFP-positive cells after transduction provides high levels of transduced granulocytes in the short term. However, using this experimental design with concomitant infusion of unselected cells and the use of oncoretroviral vectors, preenrichment of vector-expressing, transduced CD34-enriched cells did not improve long-term persistence and expression. These and ongoing studies will form the basis for clinical protocol development of gene transfer to haemopoietic stem cells in humans for the treatment of a number of genetic and acquired diseases.

The production of high-titer recombinant retrovirus is another major determinant of the efficiency of target cell transduction. Titer assessment for producer clones that contain vectors encoding proteins that can be detected using fluorescence is typically performed by flow cytometry. However, this method is both costly and labour intensive, severely limiting the number of clones that can be screened for each construct. In another report published in *Human Gene Therapy* we described a rapid, high-throughput screening method for viral quantitation of producer clone supernatant on target cells using a 96-well format. Plates were assayed using a multichannel fluorescent reader to determine the percentage of target cells expressing green (EGFP), cyan (ECFP), yellow (EYFP) or red (DsRed) fluorescent reporter genes, or their combinations. The relative fluorescence counts of target cells incubated with viral supernatant from each packaging cell clone correlated with the level of transduction, and hence, viral titer. Correlation of cell fluorescence between the fluorescent plate reader assay and flow cytometric assessment was high (r2 5 0.96). In situ titre assessment of 66 FLYRD packaging cells encoding the EGFP reporter gene identified clones (.107 colony forming units per milliliter [CFU/ml]) that provided titers up to sevenfold over the parent population. The application of this rapid, high-throughput screening method overcame many limitations imposed by the current flow cytometric screening method. This robust assay maximized the chance of identifying rare high-titer packaging clones and offers a further opportunity to optimize gene transfer protocols.

# Research funding 2002/03

**The Directors Laboratory**
"Cellular and molecular studies of the adaptive immune response in health and disease".
A Basten, R Brink, B Fazekas, C Mackay, F Mackay, S Tangye
NHMRC Program grant
2002-2006 $1,715,000

"Cell division and the regulation of immunoglobulin switch recombination at the molecular level".
C Jolly, P Hodgkin, J Hasbold
NHMRC Project grant
2001-2003 $130,000/year

"Cellular and molecular aspects of B cell responses".
T Phan
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2001-2003 $26,188/year

Ramaciotti Foundation Equipment Grant
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**Molecular Cardiology**
"Molecular studies in familial hypertrophic cardiomyopathy".
Royal Australasian College of Physicians: KPMG Fellowship in cardiovascular research.
Dr Chris Semsarian
$50,000

"Molecular studies in familial hypertrophic cardiomyopathy".
Dr Chris Semsarian
National Heart Foundation Postdoctoral Research Fellowship 2002-2003
2002-2003 $54,000/year

"Hypertrophic cardiomyopathy and sudden death"
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Dr Chris Semsarian

**Cancer Drug Resistance Group**
Centenary Institute Foundation
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Sydney Cancer Centre Foundation
$60'000

Ronald Geoffrey Arnott Foundation
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## Immune Regulation Laboratory

"The regulation and differentiation potential of human memory B lymphocytes".
PD Hodgkin and SG Tangye
NHMRC; ID 137891
2001 – 2003 $75'000/ year

"Cellular and molecular studies of the Adaptive Immune Response in Health and Diesease".
A Basten, R Brink, B Fazekas de Groth,
C Mackay, SG Tangye
NHMRC; ID 183700
2002 – 2006 $1'715 000/ year

"Lymphocytes activations and anti-tumour immunity mediated via SAP- associating surface receptors in health and disease".
SG Tangye
NSW Cancer Council; ID 115
2002 – 2004 $70'000/ year

## Liver Laboratory

"Inhibition of human anti-pig NK Cells by HLA-expression".
NHMRC CJ Martin Fellowship 2000-2003
Dr Alex Sharland

"Differential gene expression in hepatic wound healing".
NHMRC CJ Martin Fellowship 2002-2005
Dr Nick Shackel

"Role of hepatocytes in inducing primary CD8+ T cell activation and tolerance".
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Dr Patrick Bertolino

Transgenic mice: "a unique model to reassess specific T cell suppression".
NHMRC Project grant 2001-2003
Dr Patrick Bertolino

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Dr David Bowen

"Generation of a transgenic mouse model for Hepatitis".
The Cecilia Kilkeary Foundation 2002
Dr Patrick Bertolino

## Mycobacterial Research Laboratory

"Genetic adaptation of Mycobacterium tuberculosis for intracellular survival"
WJ Britton, H Briscoe.
NHMRC Project Grant
2001-2002 $88'658/ year

"New Strategies to subunit vaccines against tuberculosis".
WJ Britton, JS Triccas
World Health Organisation
2002 $US 45'000/ year

"Cell migration and granuloma formation in the expression of protective immunity against tuberculosis in the lung"
WJ Britton, B Saunders.
NHMRC Project Grant
2002 $70'000/ year

"Improved vaccines against tuberculosis based on dendritic cell manipulation".
WJ Britton, H Briscoe.
NHMRC Project Grant
2002 $85'000/ year

"Molecular analysis of host-bacterial interactions in Mycobacteria"
WJ Britton, BM Saunders, JA Triccas
Rebecca L Cooper Research Foundation
2002 $14'900.

"New strategies to vaccinate against mycobacterial tuberculosis"
WJ Britton, JA Triccas
Community Health & Anti-Tuberculosis Association.
2002 $60'820.

"Development of novel vaccine against tuberculosis".
WJ Britton, C Demangel, Thomson, I Ramshaw.
Cecilia Kilkeary Foundation.
2002-2003 $61'590

## T Cell Biology Group

"The role of dendritic cell subsets in the decision between T cell tolerance and immunity".
NHMRC Research Fellowship 2000-2004
A/Prof Barbara Fazekas

"T- dependent immune regulation in Murine and human inflammatory bowel disease".
Crohns and Colitis Foundation of America 2001-2003
A/Prof Barbara Fazekas

"A TCR transgenic model of transplant rejection and tolerance".
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Dr David Gracey

## Gene Therapy

"Improving gene transfer into haemopoietic cells".
NHMRC Project grant 2000-2002
A/Prof John Rasko

"Epigenetic Silencing of Retroelements in Mammalian Stem Cells: a role for RNA interference?".
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A/Prof John Rasko, Prof David Martin

"Identification of the genetic basis of inherited neutropenia".
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A/Prof John Rasko

"Preclinical studies of systemic gene therapy".
Anthony Rothe Foundation Postgraduate Research Scholarship2002-2004
Dr Stephen Larsen

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A/Prof John Rasko

# Publications 2002/03

**BOOKS**

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**Journal Publications**

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Ajami K, Abbott CA, Obradovic M, Gysbers V, Kahne T, McCaughan GW, Gorrell MD (2003) Structural requirements for catalysis, expression, and dimerization in the CD26/DPIV gene family. *Biochemistry* (in press)

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# Board of Governors

## Constitution

The constitution of the Board of Governors is prescribed in s 7 of *the Centenary Institute of Cancer Medicine and Cell Biology Act* (1985) (The Act). In accordance with ss (7)(2) and Schedule 1, s 4 of the Act, Governors are appointed by the Governor of New South Wales for a three-year term and may be reappointed.

### Current Appointments

*Vice Chancellor, University of Sydney or nominee, approved by the University Senate…*
**The Honourable James Longley**
Appointed as a Governor in 1997 and as Chairman in 2002. Formerly the State Member for Pittwater for almost 10 years, Mr Longley held various ministerial portfolios in the NSW Government between 1992 to 1995 and was the Chief Executive Officer of Anglican Retirement Villages, Diocese of Sydney between 1996-2000. Mr Longley is currently Senior Finance Executive with Business Development, Commonwealth Bank of Australia.

*Dean, Faculty of Medicine, University of Sydney or nominee, approved by the University Senate...*
**The Honourable John Brown AO**
Appointed as a Governor in November 2001. Mr Brown has held the position of Chairman of the Tourism Task Force since 1989. He was the Member for Parramatta in the Federal House of Representatives for thirteen years during which time he held various Ministerial portfolios including Arts, Sports, Environment and Territories. In 1987 Mr Brown was named the Australian of the Year by 'The Australian' Newspaper.

*Chair, Board of Central Sydney Area Health Service or nominee, approved by the Area Health Service Board...*
**Dr Diana G Horvath AO**
Appointed in July 1993. Dr Horvath is the Chief Executive Officer of the Central Sydney Area Health Service. Dr Horvath is a former Chairman of the National Health and Medical Research Council and President of the Australian Healthcare Association. She has served as a Commissioner of the Health Insurance Commission and as a member of the Trade Policy Advisory Council of Australia for six years.

*General Superintendent, Royal Prince Alfred Hospital or nominee approved by the Central Area Health Service Board...*
**Mr Michael Tidball**
Appointed as Centenary Institute's Treasurer in 2002. Mr Tidball was appointed as a Governor on the Centenary Institute Board in 2001 and the position he currently holds is Chief Operating Officer of the Law Society of New South Wales. He has held the title of General Manger for the following companies; Residential Age Care Anglican Retirement Villages, Legalcare Group, and Corporate Strategy Group Technicatome. In addition, he has held various Government positions such as General Manager for the Department of Business, the Arts and Sports and Tourism (ACT Government). He was also nominated as National Manager for Government Business, Minet Australia; and State Policy Advisor to the NSW Ministers for Health, Community Services and Ageing

*Persons nominated by the NSW Minister for Health...*

**Ms Sam Mostyn**

Appointed as a Governor in 2003. Ms Sam Mostyn has an extensive background in law, corporate affairs, human resources and politics. As a Group Executive of Culture and Reputation at IAG she is responsible for managing the corporate affairs, government and policy, social responsibility, and human resources functions. Most recently, Ms Mostyn was the Director of Corporate Development and Acting Human Resources Director at Cable & Wireless Optus. She also spent two years in London in the role of Group Director, Human Resources for Cable & Wireless plc. Prior to joining Cable & Wireless Optus, she was a senior adviser (communications) to the former Australian Prime Minister, The Hon PJ Keating. Ms Mostyn is legally trained and serves on the Academic Advisory Board of the Australian Institute of Management and the Board of the State Rail Authority of New South Wales. She is a Director of Insurance Australia Group Services Pty Limited, NRMA Life Limited and NRMA Staff Superannuation Pty.

**Mr John Samaha**

Appointed in 2003. John Samaha is a partner at Mallesons Stephen Jaques. He specialises in litigation, regulatory investigations and risk management strategies. In 1992 he was seconded to the Chairman's office, Australian Securities Commission as adviser to the Director of Enforcement

*Persons nominated by the Commonwealth Minister for Health...*

**Professor John Mathews AM**

Appointed as a Governor in October 2000. He is the Head of the National Centre for Disease Control, Health and Aged Care as well as a Visiting Professor of the University of Sydney. Professor Mathews formerly held the Inaugural Directorship of the Menzies School of Medical Research in Darwin and has been a member of numerous advisory and review groups for the NHMRC and Federal Government.

**Mr Alastair Davidson**

Alastair Davidson is Managing Director of Aurora Funds Management based in Sydney. Prior to this he has held executive positions in the banking and financial services industry for 15 years in the UK, USA and Australia. This included Salomon Smith Barney in Sydney for eight years as co-head of its new product group, specialising in equity derivatives. He is a member of the Institute of Chartered Accountants in Scotland.

*Persons elected by the nominated governors…*

**Mr Paul Harris**

Appointed to the Board in May 1998. He is a member of the Australian Stock Exchange Ltd and the Securities Institute of Australia. Mr Harris has worked in the securities market in Australia for nearly 30 years holding a number of senior positions in merchant banks and stockbrokers. He is currently a Director of various companies including Ten Group Ltd, Gresham CEA Management Ltd, Gresham Technology Management Limited, ABN AMRO Australia, Hoare Govette (Securities) Ltd and Stadium Australia Club Ltd. Mr Harris was appointed Chairman of Bundanon Trust in 2000.

**Ms Jan Hogan**

Appointed as a Governor in May 1998. She is a Psychologist and Family Therapist with an active practice and Director of Solution Focused Counselling Centre. Ms Hogan holds directorships in several private companies.

**Mr Malcolm Noad**

Formerly Chairman of Board of Governors from May 1999-2002, Mr Noad is Managing Director, Nationwide News, News Limited and has been a member of the News Limited Board since 1992. In addition, Mr Noad is Chairman of the Board of the National Rugby League and a member of the Boards of Herald and Weekly Times Newspapers, Davies Bros, Advertising Newspapers and Community Newspapers in Perth as well as being a Trustee of the Centenary Institute Medical Research Foundation.

## The Director…

**Professor Antony Basten AO**

Appointed Executive Director in May 1989. He holds the Chair of Immunology at the University of Sydney, is a consultant physician at Royal Prince Alfred Hospital, is Director of the Central Sydney Area's Clinical Immunology & Allergy Service and Chief Scientist of CenTec Limited.

# Institute staff 2002/03

**Director**
Prof Anthony Basten AO FAA FTS MBBS Dphil(Oxon) FRCP FRACP FRCPA,

**General Manager**
Denyse Bartimote

**CenTec**
*CEO* Dr Paul Tan

**Finance**
*Manager* Elaine Cook
*Accountant* Viraf Variava
*Accountants Administration* Dana Kilanis

**Office Staff**
*Business Development Manager*
Dr Nicholas Pearce BSc (Hons) PhD
*Human Resource Manager* Judith Barry
*External Affairs Manager Kate* Scott
*Research Support Officer* Sonja Bates
*Personal Assistant to Director*
Gabriella O'Neil
Personal Assistant to General Manager
Ruth Godfrey
*Receptionists* Dinah Utian, Maisie Aguliar, Sonja Nichols

**Technical Support**
*Facilities & Resource Manager*
Jeff Crosbie
*Network Manager* Brian Bulliman
*IT Support Officer Adrian* Smith
*Building Service Officer* Joe Ayoub
*OIC Flow Cytometry* Joseph Webster
*Research Assistant/ Flow Cytometry*
Tara McDonald
*Librarian* Mary Linnane

**Animal Facility**
*Unit Head* Dr Jenny Kingham
*Technical Officer* Marisa Mourelle
*Technical Assistants* Catherine Sorokine (until Sept 2003), Bradley Harper, Joel Robertson
*Animal Attendants* Rachel Nowell, Catherine Sorokine, Joel Robertson (until Sept 2003), Peta Pippos, Andy Hall, Tamara Lancaster, Andrej Susor, Dane Millanta, Artika Autar
Laboratory Assistant (Media Prep) Hai Nguyen
*Work Experience* Phillip McDonagh, Katherine Barna (TAFE)

**Autoimmunity Group**
*Unit Head* Dr Alan Baxter, MBBS PhD, RD Wright Fellow
*Senior Research Officer* Dr Sean Riminton
*Research Officer* Dr Mu Yao
*Research Assistants* Margaret Jordan, Rama Kandasamy, Jian Li, Tim Butler
*Technical Officer* Tom Davis
*PhD Scholar* Christine Hawke

**B Cell Biology Group**
*Unit Head* Prof A Basten, AO FAA FTS MBBS Dphil (Oxon) FRCP FRACP FRCPA,
*Business Development Manager*
Dr Nicholas Pearce BSc (Hons) PhD
*Senior Research Officers* Dr Robert Brink, Dr Chris Jolly
*Postdoctoral Fellow* Dr Pablo Silveira
*Research Officers* Dr Pearly Harumal, Dr Dehua Chen, Dr Didrik Paus, Dr Mu Yao (Centec), Dr Adrian Grech (Aug 2003)
*Research Assistants* Michelle Amesbury, Sandra Gardam, Kamila Nejedly, Tyani Chan
Adrian Grech (until July 2003), Jian Li, Joanna Raftery
*Technical Officers* Chris Brownlee, Tom Davis (Centec)
*PhD Scholar* Marilyn Thien, Dr Tri Phan, Adam Cook, Dr Lye Lin Ho
*Work Experience* Daniel Gorman

**Cancer Drug Resistance**
*Unit Head* Dr John Allen, Senior Research Officer, BSc (Hons) Mqual (Psychology) Phd.
*Research Assistant* Tara Kanabe, Tracy Murray
*PhD Scholar* Peter Tobin
*Honours Student* Tracy Murray
*Work Experience* Emily Tu

**Gene Therapy Group**
*Unit Head* A/Prof John Rasko, BSc (Med), MBBS (Hons), PhD, FRCPA, FRACP
*Research Officers* Dr David Lu, Dr Charles Bailey, Fiona Battah, Rebecca Read
*Research Assistants* Cynthia Ng, Michelle Pedler (until August 2002)
*PhD Scholars* Vanessa Gysbers, Stephen Larsen, Keefe Chng, Bronwyn Green (until August 2002),
*Honours Students* Joey Lai, Sooan Choi, Brandon Aubrey, Stephen Gore

*Visiting Scholars* A/ Prof Heng Feng Seow, Dr Marc Dahlke, Simon Potter (until Mid 2003)
*Visiting Researcher* Rosetta Martiniello

### Immune Regulation Group

*Unit Head* Prof A Basten, AO FAA FTS MBBS Dphil(Oxon) FRCP FRACP FRCPA,
*Senior Research Officers* Dr Daniel Sze (RPA), DR Stuart Tangye
*Research Associate* Dr Joy Ho (RPA)
*Research Assistants* Danielle Priestley, Nathan Hare, Melinda Jeffels (RPA), Shi-Hong Yang (RPA)
*PhD Scholars* Elissa Deenick (until March 2003), Cindy Ma, Vanessa Bryant, Kim Lee Good
*Honours Student* Julia Ellyard

### Liver Immunobiology

*Unit Head* Prof Geoffrey W. McCaughan, MBBS (Hons) FRACP PhD, *Director AW Morrow Gastroenterology and Liver Centre.*
*Senior Research Fellows* Dr Mark Gorrell, Dr Alex Bishop, Dr Patrick Bertolino
*Postdoctoral* Dr Devanshi Seth, Dr Heather Knott
*Research Assistants* Katerina Ajami, Maggie Wang, Dr Chuanmin Wang, Lauren Holz, Jian Li, Seven Guney, Dr Nicholas Shackel (until Aug 2002), Vannessa Holohan
*PhD Scholars* Joohong Park, Shaun Cordoba, Denise Yu, Jane Huang, Sunmi Song, Amany Zekry
*Honours Student* Peter Tran
*Work Experience* Wimonrat Hoasadawut, Ricky Lim, Zeina Awad

### Mycobacterial Group

*Unit Head* Prof Warwick Britton, FRACP FRCP BSc Med (Hons) PhD
*Senior Research Officer* Dr Helen Briscoe, Dr Jamie Triccas, Dr Bernadette Saunders, Dr Grant Shoebridge, Dr Caroline Demangel (until Feb 2002)
*Postdoctoral* Dr Rowan Williams (RPA Until Sept)
*Research Assistant* Joanne Spratt, Stephen Tran, Teresa Wozniak, Umaimainthan Palendira, Meera Karunakaran (until Aug 2002)
*Technical Officers* Nathan Field, Jason Compton
*PhD Scholars* Umaimainthan Palendira, Gabriella Scandurra, Rachel Pinto, Dr Suran Fernando, Anthony Ryan, Teresa Wozniak
*Work Experience* Joanna Sutton

### T Cell Biology Group

*Unit Head* A/Prof Barbara Fazkas de St. Groth, BSc (Med) MBBS (Hons) PhD, Senior Research Fellow
*Senior Research Officers* Dr Carl Power (Until Nov), Dr Adrian Smith, Dr Nabila Seddiki
*PhD Scholar* Alex Spencer, David Gracey, Sioh-Yang Tan, Ben Roediger
*Honours Student* Caroline Higgins

### Molecular Cardiology

*Unit Head* A/Prof Chris Semsarian (RPA) MBBS PhD FRACP
*Research Officer* Dr Tatiana Tsoutsman
*Cardiovascular Genetics Co-ordinator* Jodie Ingles
*PhD Scholar* Alessandra Doolan
*Honours Student* Lan Nguyen
*Work Experience* Andrea Neal