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THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

ISSUE 6 · MICHAELMAS 2007

Contents

Editorial

This has been an eventful period for the Dunn School. Sadly, we lost two long-serving staff members – Laurence Turley and Mike Puklavec – both in the prime of their lives (see obits below). Both were dedicated contributors to the science and life of the Dunn School, and will be sorely missed.

On the positive side, we are very pleased to report that Siamon Gordon has been elected to a Fellowship of the Royal Society. We are also delighted that Elizabeth Robertson (Wellcome Principle Research Fellow) and Elizabeth Bikoff have joined the department, and we welcome Stephen Bell, formerly of Cambridge University, as our newly elected Professor of Microbiology. While pleased to announce that Gillian Griffiths has been awarded a prestigious Wellcome PRF, we are sorry that this has taken her away from us to Cambridge University. We wish her and her family great success there. Our congratulations go to Michael Ginger elected to a lectureship in Lancaster University, Ariel Blocker to a senior lectureship at Bristol University and to Paul Fairchild on his appointment to a Research Councils UK Academic Fellowship.

We wish to thank Pam Woodward and Christine Holt on their retirements. They have been at the heart of Dunn School life in the main administrative office. Their friendliness, warmth (and flower designs), provided a great welcome to visitors who could immediately sense the happy environment they were entering. Also amidst those recently departed is Colin Ryde, the Departmental Administrator for the past 13 years, who has moved to the new Chemistry Department. I am grateful for what Colin achieved over his years with us.

On the "development" side, I am pleased that we have been able to endow a Chair in honour of Cesar Milstein, the inventor of monoclonal antibodies. So many scientists in this department have benefited from Cesar's discovery and indeed training, that this is as good a way of saying thank-you as one could imagine. Celia Milstein, patron of our appeals committee, has been very supportive of this development, and I am grateful to her and the other members of the Fund-raising committee (Claudio Cuello, Sir Greg Winter, George Brownlee, Salvador Moncada, Paul Langford, Susan Harrison) for their dedicated efforts in making the Chair possible. This Chair will provide the Dunn School with the opportunity to attract a world-class scientist to Oxford, at a time when the Oxford community are making a major commitment to enhancing its cancer research effort.

Endowments of this kind are the key to enabling the department to establish financial stability, and we are now in the fortunate position that 5 of our 7 chairs are covered by endowments. In the longer term we will seek support to endow the remaining Chairs, and to establish a long-overdue Chair in Immunology.

Our graduate training programmes continue to evolve rapidly under the energetic management of Anton van der Merwe and support of Lucinda Risius. We now host 80 graduate students in the department, and our studentships are very competitive. The latest addition to our list is one established in honour of Norman Heatley to provide training in microbiology. We are very grateful to Eric Lax, Susan Harrison, Merck Sharpe and Dohme, and indeed the Heatley family and friends who have made this endowed studentship possible.

The coming years will see appointments to a number of new senior positions as our senior colleagues reach retirement. This will be an interesting and challenging period for the department. Finally it will not surprise readers to learn that I enthusiastically endorsed the editors proposal to take an immunological theme for this edition of Fusion.

Herman Waldmann

News

Professor Siamon Gordon

Honours

Professor Siamon Gordon has been elected to a Fellowship of the Royal Society

The citation is: **Professor Siamon Gordon** is distinguished for discovering new macrophage-restricted plasma membrane antigens and receptors and demonstrating their functions in differentiation, adhesion, phagocytosis, immune activation and secretion. These surface molecules are important in innate immunity to microbial and fungal infection, in tissue homeostasis and in pathogenesis of a range of inflammatory and metabolic diseases.

Awards

We are delighted that the following laboratories have all won research funding of more than £100,000.

Brownlee	MRC
Barclay	MRC
Proudfoot	BBRC
Macpherson	Pfizer
Murphy	Wellcome Trust
Vaux	Synaptica
Powrie	Wellcome Trust
Cook	EPA Research Fund
Fodor	European Commission
Norbury	Cancer Res UK
Sattentau	European Commission
Fairchild	Geron Corporation

Long Service

Congratulations to **Steve Simmonds** on achieving 40 years uninterrupted service

NB. of those still seen regularly in the Lab only Sir Henry Harris (first appearance in 1952) and Eric Sidebottom Jan 1966 arrived in The Dunn School before Steve. Peter Cook arrived in Sept 67, Gordon MacPherson in April 68, Simon Hunt Oct 69 Sue Humm in June 70 and Stephen Clark in Sept 70.

Prizes

Herman Waldmann 2008 Thomas E. Starzl Prize in Surgery and Immunology."The committee voted unanimously to award you the prize based on your outstanding achievements in Immunology and the major impact these achievements have had on organ transplantation". Established in 1996, the Starzl Prize has been awarded to 13 international leaders in organ transplantation and immunology. These include Paul Terasaki, Sir Gustav Nossal, Francis Moore, Rolf Zinkernagel, and Sir Roy Calne, among others. The winners of the Starzl Prize are invited to Pittsburgh to present a lecture and receive the Prize.

Mick Dye, a Medical Sciences Division Research Prize.
Kathy Lui, the Peter Beaconsfield Prize in Physiological Sciences.

Retirements

Pam Woodward, Pam first joined the lab in Nov 1965, but then took time out to look after her children. However during this time she was still available for 'contract work' and indeed she typed one of your editor's DPhil thesis in 1969. She re-joined in Sept 1975 and has been with us since then.

Christine Holt joined the lab in Jan 1994 and after 13 years excellent service, particularly to Siamon Gordon, left almost silently in July.

Moves

Gillian Griffiths (to Cambridge University)
Colin Ryde, (to Chemistry)
Michael Ginger (to Lancaster University)
Ariel Blocker (to Bristol University)

Immunology in the Dunn School

Alan Williams

In the summer of 1991, Alan was elected to succeed Sir Henry Harris as the Professor of Pathology and Head of the Dunn School. Although perhaps surprising to the establishment, this was generally seen as an imaginative and exciting appointment. In characteristic fashion Alan set about making ambitious plans to strengthen research at the Dunn School and move it technologically into the 21st century. Who of us could, at that time, possibly imagine that Alan would not survive long enough to take up the Chair?

His tragically early death from lung cancer at the age of 46 in April 1992 deprived Oxford and the whole scientific world of one of its most productive and promising scientists. It also deprived his family and many colleagues of a true and trusted friend and adviser.

His citation on election to the Royal Society in 1990 sets out clearly the impact he had already made in virtually establishing a new field in molecular immunology.

Williams is a pioneer and recognised authority in the rapidly expanding field of leukocyte differentiation antigens. He made leading contributions to the development of immunological and biochemical methods used for the characterisation and subsequent isolation of cell surface molecules. His purification and sequence analysis of Thy-1 antigen (together with parallel studies on transplantation antigens by others) established the general approaches later applied to many other molecules. Subsequently he was the first to use monoclonal antibodies for those purposes. His early discovery that the Thy-1 gene was evolutionarily related to the immunoglobulin genes was fundamental to his development of the concept of an immunoglobulin super-family.

Alan was born in Melbourne in 1945, the second child in a working class family of six. His father was described as quiet and reflective, his mother as flamboyant. Both were deeply committed members of the Salvation Army and Alan played the cornet in the brass band; the source of his lifelong interest in music.

Although not apparently a 'star' at school he went on to Melbourne University to read Agricultural Science. Here he blossomed and was invited to stay on as a graduate student. However after a short placement with Bede Morris (a Dunn School Alumnus) at the John Curtin School at the ANU in Canberra, Alan decided to work for a PhD with Bill Elliott in the Dept of Biochemistry at Adelaide. This was a very productive period in which he learnt sound biochemical research techniques and fostered an interest in cellular development programmes. Elliott (who had just spent a sabbatical in Rod Porter's lab in Oxford) encouraged Alan to move to Oxford for his 'post-doc' and after first arranging to work with John Gurdon, for practical reasons Alan transferred to Rod Porter's lab where his interest in molecular immunology quickly developed. His first challenge here was to search for 'IgT', the hypothetical immunoglobulin T cell receptor with Jens Jensenius. After several years of chasing the 'will-o-the-wisp' they were convinced that the receptor was not an immunoglobulin and that they had discredited the theory but it took others another 10 years to find the real T cell receptor. Alan moved on to work on isolating and characterizing the rat Thy-1 antigen, using techniques developed from those of Mike Crumpton at NIMR. In contrast to the IgT work this was strikingly successful. The relatively large amounts of material available allowed notable chemical and physical studies which resulted in a comprehensive picture of the structure of the antigen. The site and tissue-specific patterns of glycosolation, the method of attachment to the cell membrane via a 'GPI' anchor, and the similarity of amino acid sequence to the V region of immunoglobulin

molecules were all new and exciting findings. The last especially, occupied a good deal of Alan's intellectual energy and led to what is generally thought to be his most important conceptual advance; that of the Immunoglobulin Superfamily.

At the same time Alan was one of the first scientists to capitalize on Milstein & Kohler's 1975 discovery of a method to make monoclonal antibodies. This was a veritable gold mine in the search for lymphocyte surface molecules and the extent of its success is set out in the book on which Alan worked until the day before his death, "The Leucocyte Antigen Factsbook"; and in his other 152 scientific publications.

Although Alan's scientific progress evolved more or less seamlessly from his arrival in Oxford in 1970, it was greatly influenced by his appointment in 1977 as Director of the MRC Cellular Immunology Unit situated in The Dunn School. The Directorship had become vacant on the appointment of Jim (later Sir James) Gowans as Secretary of the MRC. In the words of his Royal Society obituarist, Michael Crumpton, Alan was at that time "riding the crest of a scientific wave" and over the next decade "he grew from being a talented, forthright and outspoken young man to an exemplary leader with broad prospectives, readily assuming the mantle of responsibility". In his direction of the unit he was greatly assisted by Don Mason and Neil Barclay who became Alan's trusted lieutenants. An increasing flow of graduate students, postdocs and senior visitors came to work in the Unit as its reputation spread. It was a lively and rewarding place to be.

Alan may have been described at various times as argumentative, blunt, single-minded and prejudiced, but also as honest, dedicated, perceptive and visionary. There is universal agreement that he was a very gifted scientist cruelly cut off in his prime.

The legacy of the immunoglobulin superfamily

Neil Barclay

Almost 20 years ago the concept of the immunoglobulin superfamily as a group of proteins with particular suitability for recognition events was well established and summarised in a key review (Williams and Barclay 1988 *Ann Rev Immunol* 6:381). Since then the concept of superfamilies of cell surface domains has been central to understanding the role of the lymphocyte cell surface and this has been carried on in the Sir William Dunn School of Pathology. As is so often the case, it is technical advances that have proven to be central to progress and some of the key areas are highlighted in this article.

Interactions of the surface proteins of lymphocytes

The seminal review of the leukocyte cell surface initiated by Alan Williams but only completed after his death in 1992 (Barclay et al; The Leucocyte Antigens Factsbook, Academic Press) spelt out the complexity of the surface of leukocytes in terms of the types of proteins they expressed. The majority of proteins found solely on lymphocytes which are likely, therefore, to mediate immunological functions, were found not to be enzymes but proteins capable of being recognised by other proteins. Immunoglobulin superfamily (IgSF) domains were particularly common. Many seemed likely to interact with other cell surface proteins and these have been a major focus in recent years. A significant breakthrough was the introduction of the BIAcore™ technology in which protein interactions could be studied in real time without the need to use labels such as radioactivity. This, coupled with methods to produce large amounts of recombinant proteins corresponding to the

extracellular regions of the leukocyte surface proteins, enabled these interactions to be studied quantitatively in a novel way. The first interaction studies between CD48 and CD2 showed that these interactions could be very weak with affinities in the 10-100μM range and this set the paradigm for most other interactions between these classes of molecules. The relevance of kinetics was studied in detail by Anton van der Merwe, who, in collaboration with Simon Davis (who had, by then, moved to the Nuffield Department of Medicine), developed a theory of how T cells respond to antigen involving the movement of lymphocyte proteins when the cells come in contact with antigen presenting cells (known as the kinetic segregation model; see *Fusion 5* page 10).

Identification of new interactions

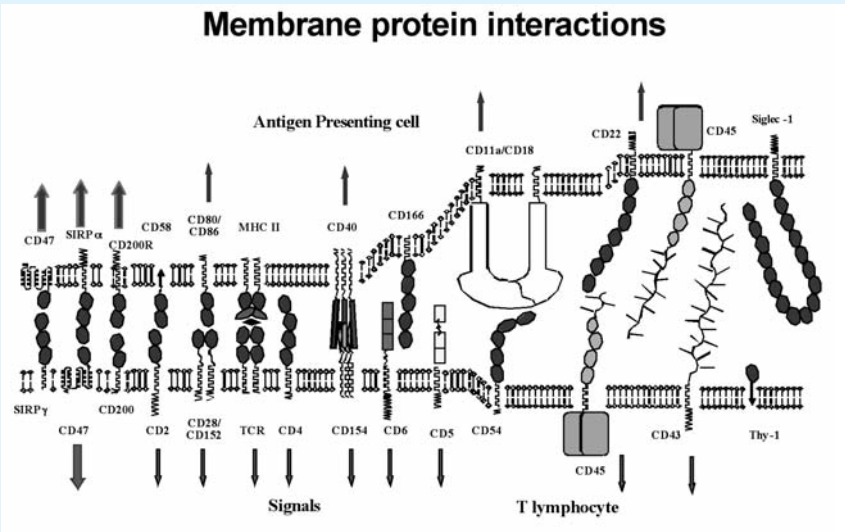
The finding that the interactions between cell surface proteins were much weaker than expected and that the proteins had particularly fast dissociation rates i.e. half lives of around one second, made searching for new ligands difficult. Nevertheless, new technology, developed by Marion Brown using multivalent beads, has allowed several new interactions to be identified and analysed. Some of the interactions characterised are illustrated in the cartoon that includes well known interactions such as the T cell receptor with MHC antigens and integrins with CD55 (the IgSF domains are shown as ovals).

Structures of the surface proteins

The development of good expression systems in the early 90's provided large amounts of recombinant protein suitable for analysis of structures by X-ray crystallography. Using methods to simplify the glycosylation of the proteins Simon Davis, with the crystallography group of Dave Stuart in Molecular Biophysics determined the structure of CD2, the first adhesion protein to be characterised and not surprisingly an IgSF domain. Further structures included part of CD4 and more recently the ligand binding domain of signal inhibitory protein (SIRP) alpha, a macrophage IgSF receptor that recognises another IgSF protein CD47. Interestingly, this binds in a different manner to CD2. Whereas CD2 binds through one of the faces of the domain like many interactions between IgSF cell surface proteins, SIRP· binds more like an antibody or T cell receptor through the loops at the end of the domain providing a recognition system that is sensitive to small changes in sequence: this provides the molecular explanation for the fine specificity of the SIRPs that are members of closely related families of proteins called 'paired receptors'.

Quantitative analysis of signals generated.

One of the legacies of Alan Williams and, indeed, of the late Rodney Porter, with whom both Alan and I worked in the 1970's, was an appreciation of quantitation. In addition to the quantitative analysis of interactions of the extracellular regions of the leukocyte proteins and consideration of concepts such as separation of the cells, abundance of the proteins and their post-translational modification, recent studies have begun applying this rigour to the inside of cells. Clearly a major role of many of the surface proteins is to give or enable signals to be transmitted to cells expressing the receptor. Many of the adaptors, kinases, phosphatases and other proteins involved in transmitting signals or linking with the cytoskeleton can bind more than one substrate. Clearly a quantitative analysis is required to work out the hierarchy of interactions and this is now a major new focus for Marion Brown's research. What is surprising is that so little of the published work on such interactions is analysed at physiological temperature!



The Legacy and the Future

One other major line of research in the MRC Cellular Immunology Unit involved linking biochemistry with cellular immunology, the main focus of Don Mason's research. The introduction by Alan of monoclonal antibodies capable of recognising new cell surface proteins, transformed cellular analysis and led to many seminal findings, from the first description of CD4 as a marker of T cells, to the splitting of the T cells into subpopulations that included a population that could control the others – the start of the regulatory cell concept that has exploded in recent years. This work is currently being continued by Fiona Powrie and Kevin Maloy in the very same space once occupied by Don. Monoclonal antibodies still remain powerful reagents and continued to be made by Mike Puklavec until his untimely death. The principle that the monoclonals would be available to researchers on publication has been maintained with the result that these are standard reagents worldwide – and the world famous OX series has now reached OX130. The impact of Alan Williams continues to be felt, both because his publications are still highly cited (144 times in 2006), maintaining his remarkable citation record (averaging over 150 citations per paper) and because of the impact in the field of immunology the Dunn School continues to have.

Reprogramming the Immune System

Steve Cobbold

Therapeutic Immunology Group

The only treatments currently available for patients with autoimmune diseases or after organ transplantation provide little more than symptomatic relief, by non-specifically suppressing the whole immune system. The side effects of such immunosuppressive drugs include increased risk of infection and cancer, and even then they are not always effective, leading to disease relapses and graft rejection. Back in 1986 we showed that foreign proteins could be accepted by the immune system of an adult mouse, as if they were "self", by giving them under the cover of a brief treatment of a monoclonal antibody (mAb) against the CD4 molecule found on the surface of thymus derived lymphocytes (T cells). We later demonstrated that similar, short treatments with non-depleting, but functionally blocking, mAbs against various T cell surface molecules could induce life-long acceptance of tissue or organ grafts. It was these series of experiments that first clearly established "reprogramming" of the adult immune system as a therapeutically obtainable goal.

Short-term treatment for long-term benefit

Over the next 20 or so years, the Therapeutic Immunology Group (TIG), and the Therapeutic Antibody Centre (TAC), under the leadership of Prof. Herman Waldmann, worked together to develop and test, with the help of many clinicians around the world, appropriate mAbs to reprogram the immune system of humans, in clinical situations. The first generation of such mAbs was called CAMPATH. These mAbs deplete lymphocytes, allowing the immune system to regenerate and, in some cases, reset itself. CAMPATH was also found to be useful for treating certain types of chemotherapy-resistant leukaemia, and this is what it is now licensed and marketed by, by a major pharmaceutical company. In addition, it is still being tested for its ability to reprogram the immune system in multiple sclerosis and in recipients of transplants. Second generation mAbs, such as a non-activating anti-CD3, are currently being tested for immune reprogramming in autoimmune diseases, such as type 1 diabetes.

From bench to bedside and back again

Our direct involvement with the clinical application of mAbs has now waned, as the costs to run larger, and more regulated, clinical trials have increased to the point where only large pharmaceutical companies can sustain them. The TAC has now relinquished all the clinical development to industry, and has pursued other areas that are currently more appropriate for an

academic centre, such as vaccine development. What has become clear, however, is that successfully extending immune reprogramming therapies from animal models to human clinical situations, depends on a much broader understanding of how the immune system is regulated in both health and disease. In particular, we now recognise that clinical situations are complicated by other factors, such as the infectious history of the patient and the concurrent use of other medications, which may interact or block attempts to achieve immune reprogramming. This all means that our main focus has shifted back to the basic mechanisms of immune regulation, which can only be fully investigated in animals where we can safely model the various factors that we have learnt are a barrier to immune reprogramming in the clinic.

Current research: it's all about regulation

Immune tolerance, until the early 1990's, was considered to depend entirely on the clonal deletion of potentially reactive T cells during development in the thymus. Only in the past 10 years or so has it become clear that tolerance induced through immune reprogramming in the adult is dependent on regulatory T cells (Tregs). Our current focus is therefore to determine the mechanisms by which Tregs are induced by mAb treatment, and how they work to reprogram the immune system, particularly in the acceptance of foreign tissue grafts. We have recently demonstrated that transforming growth factor beta (TGFβ,) is always essential for the generation of new graft-specific Tregs, even if the therapeutic manipulations used to generate tolerance are quite different. Whether we use therapeutic mAbs as above, or specialised, tolerogenic donor-derived cells (modulated dendritic cells), we generally find Tregs are generated and concentrated within the tolerated graft tissue. This is leading us to investigate how Tregs interact with, and influence the properties of, both the grafted tissue itself and the dendritic cells that infiltrate it.

Privileged to be off drugs

Historically, a state of immune privilege was used to explain why certain organs, such as the eye, testis, brain, and the foetus, were generally "less rejectable" by the immune system. Very recently, there has been a convergence of data and ideas that suggest that the tolerance induced to transplants and the mechanisms of immune privilege are both the consequences of a localised interaction between the tissue with infiltrating dendritic cells and Tregs. We are starting to find that Tregs are able to turn on a protective gene expression profile when recognising donor-derived dendritic cells and in tolerated tissue grafts, which further amplifies the tolerogenic microenvironment such that any new, non-tolerant T cells that enter the graft are suppressed from rejecting, and may even be converted to Tregs themselves (a process we call "infectious tolerance"). Our aim now is to understand this tolerogenic microenvironment, and eventually how it may be influenced by the various factors we found to be a limitation to clinical application of immune reprogramming, such as memory T cells (acquired via infections or after immune depletion) and immunosuppressive drugs. In the process of defining and understanding the tolerogenic microenvironment we should also be able to develop clinical tests (biomarkers) that would indicate whether treated patients can develop sufficient immune tolerance to allow selective reductions, or even cessation, of immunosuppressive drugs.

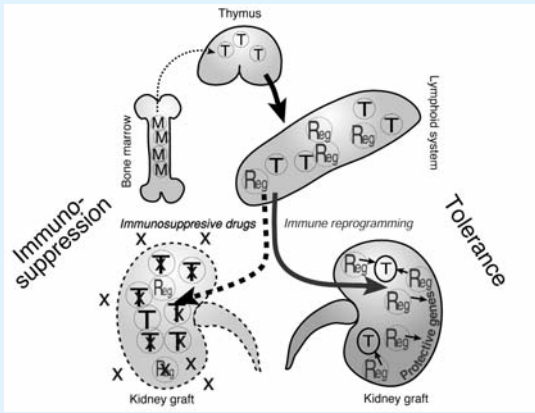
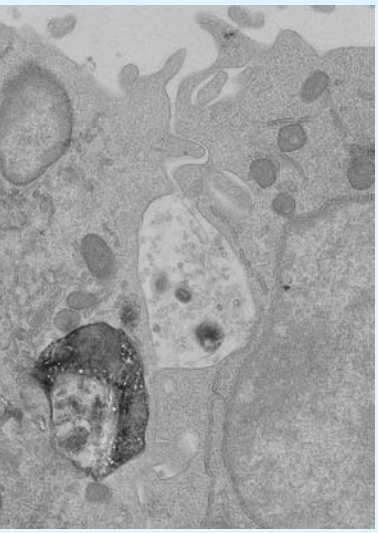


Figure legend

T cells (T) develop in the thymus from bone marrow stem cells (M). The normal lymphoid system contains a balance of potentially aggressive T cells and regulatory T cells (Reg). In patients given a kidney graft, conventional immunosuppressive drugs are used to control the aggressive T cells, but these same drugs may also compromise regulatory T cell activity. After immune reprogramming, however, regulatory T cells specific for the kidney graft predominate and naturally control any locally aggressive T cells. The regulatory T cells also induce protective genes within the graft that help to maintain the tolerant state.

Probing the Immunological Synapse

Misty Jenkins recently joined Gillian Griffiths and her team after completing her PhD in the Department of Microbiology and Immunology at the University of Melbourne, Australia. Here she gives a flavour of current research within her new laboratory.



e.m. of immunological synapse (Jane Stinchcombe)

The presence of infectious microorganisms such as viruses, parasites and bacteria has driven the evolution of specialized and complex immune responses to protect the host from infection. An effective immune response relies on specialized immune cells to specifically recognize foreign antigens, which are presented on the surface of antigen presenting cells.

CD8+ T lymphocytes (CTL) are one immune cell which plays a crucial role in the acute control of many infections, killing targets mainly via the release of cytotoxic proteins which induce cell suicide. The cytotoxic proteins are stored within secretory lysosomes and contain a number of toxic proteins including a spectrum of serine proteases (granzymes), the pore-forming protein, perforin, as well as other ubiquitously expressed lysosomal proteins. Following antigen recognition, T cells polarize their secretory machinery towards the target cell, and secrete the constituents into the tight junction between the two cells, known as the immunological synapse (see Figure).

In addition to CTL, there are other cell types which utilize secretory machinery, notably the melanocyte, which secretes melanin, giving rise to the pigmentation in skin and hair. Remarkably there have been diseases identified in which secretion from both CTL and melanocytes is impaired, suggesting a common secretion machinery. Given this shared phenotype, rare human genetic diseases which result in a combination of albinism and immunodeficiency have been identified. By studying mutations which give rise to these diseases, the Griffiths laboratory has successfully identified novel proteins required for secretion of lysosomal compartments in both melanocytes and CTL. Using CTL clones, in which the delivery of lytic granules is impaired at different stages of secretion, the lab has identified mechanisms of secretion at the immunological synapse.

Polarisation of secretory lysosomes is initiated by the movement of the microtubule organizing centre (MTOC), focussing microtubules towards the immunological synapse. Recently, the lab has shown docking of centrioles at the site of CTL-target synapse formation, facilitating the delivery of lytic granules to the secretory cleft. Further studies are focusing on additional proteins involved in secretion, including those which allow the sorting of cytotoxic proteins to their compartments, allow granules to migrate along microtubules, and to dock and fuse with the plasma membrane. By studying the role of the actin cytoskeleton and the trafficking of intracellular vesicles, we hope to elucidate the molecular mechanisms which generate synapse formation and subsequent T cell activation. Further interesting observations made by the laboratory include the acquisition of target cell membrane by the CTL as the two cells release their synapse. Current research in the laboratory is also concentrating on understanding the microenvironment of the immunological synapse. This research is providing powerful insights into the working of the cell, and may identify novel targets for therapeutic intervention. This research also provides an excellent illustration of the power of combining confocal imaging, electron microscopy, biochemical analysis and immunology to understand membrane-cytoskeleton interactions. As such, the Griffiths laboratory provides an excellent link between immunologists and cell biologists, essential for determining the molecular machinery required for immune cell function. In turn, a deeper understanding of the qualitative factors which govern CTL cytotoxicity will allow an enhanced dissection of cell mediated immunity, essential to aid the development of therapeutic intervention, when these cells fail to function properly.

Uncovering the Mysteries of T Cell Signalling

Dhaval Sangani

The early events in a T lymphocyte following the engagement of a T cell receptor (TCR) by an appropriate peptide-MHC complex include rearrangement of cell surface molecules, phosphorylation cascades and activation of downstream signalling assemblies. The plasma membrane of the T cell during this process is not simply a passive repository of proteins and lipids, but is an active platform for assembling and harboring multimolecular signalling machinery critical for an immune response by the T cell. Thomas Harder's laboratory studies the role of membrane domains and multiprotein signalling assemblies in transduction of the signal from the TCR to the cell interior. These two elements can be broadly described as lipid-based interactions and protein-based interactions in the T cell plasma membrane.

To study lipid-based interactions, Tobias Zech, a graduate student in the lab uses the lipid dye 'Laurdan' (which changes its fluorescence emission spectrum with polarity of the environment) to image areas in the vicinity of the engaged TCR to understand the change in the membrane architecture upon T cell activation. He finds that the condensation of the membrane at the site increases upon TCR triggering, probably reflecting the formation of membrane microdomains. He further asks whether disrupting these domains affects T lymphocyte activation. Disruption of membrane condensation is achieved by introducing into the cell membrane an analogue of cholesterol called 7-ketocholesterol (7KC) which hinders close packing. Treatment of Jurkat T cells with 7KC, while not affecting the qualitative nature of protein assemblies post triggering, drastically reduces the quantity of proteins recruited to the activation site, presumably by disrupting membrane condensation. The biophysical description of a large scale change in membrane texture upon arrival of a signal is an important finding towards understanding the nature of activation signals for a T lymphocyte.

Our ongoing research includes 'lipidomics' of the TCR-signalling domains, isolated by the technique of immunoisolation, and a proteomics approach to identify and determine the function of novel protein players in the activation of T lymphocytes. Immunoisolation is a technique developed by Thomas Harder, which involves coating a tiny magnetic bead with TCR activating antibodies, encouraging the formation of conjugates with Jurkat T cells and mechanically homogenizing the cells to retrieve plasma membrane patches enriched in the TCR signalling machinery. Lipidomics performed on these samples has revealed that the chemical composition of lipids is a dynamically-changing parameter, with some compositions (such as tightly packed saturated lipids and cholesterol) being preferred for housing active signalling protein machineries. This represents the first direct biochemical evidence in support of the existence and role of membrane microdomains in signalling.

My own research is aimed at understanding the mechanistic basis for the construction of multiprotein assemblies formed immediately after the engagement of the TCR in the plasma membrane. The key player in this event is the transmembrane adaptor protein LAT which is phosphorylated on multiple tyrosines upon TCR triggering. The phosphorylations on LAT then act as docking sites for various SH2 domain containing adaptors like Grb2, Gads and enzymes like PLC-γ1, Vav, PI3 Kinase, Sos and Itk. These LAT-nucleated protein-protein interactions are crucial to the integration and transmission of the signal for further distal events and the optimal outcome of T cell activation. The adaptor protein Grb2 (which is known to bind to distal three phosphotyrosines on LAT), is a major species found in the proteomics studies of TCR/LAT-nucleated membrane domains. Earlier studies from the lab and others have revealed a cooperative

mechanism of recruitment of Grb2 and PLC-γ1 (which binds one of the phosphotyrosines of LAT) following TCR triggering. Partially reconstituting LAT-Grb2 assemblies in artificial supported bilayers would facilitate probing the stochiometries, lateral organization and diffusion behavior of LAT-Grb2 oligomers. Towards this end, an insect cell based expression system for producing recombinant, phosphorylated, membrane anchored LAT has been designed. Recombinant LAT from such a system would be employed in experiments to ask what effect the membrane composition has on the nature of protein assemblies and vice-versa. Medical intervention by small molecule drugs targeted against LAT-Grb2 or other such LAT-based interactions is a tempting avenue to engineer the response of a T cell and curtail autoimmune disorders.

Development news

We should like to re-iterate the good news reported in the editorial that sufficient funding has now been obtained to endow a Chair of Molecular Cancer Biology in honour of Cesar Milstein and also a Norman Heatley Studentship to provide a training in microbiology for a graduate student. Professor Waldmann has listed those principally responsible for the successful fund raising. We are very grateful to them all.

Lou Angelou

We are delighted to welcome **Lou Angelou**, a new Development Officer with special responsibilities for the Dunn School.

New Service

Oxford Module Consortium; to provide libraries of reagents; DNA constructs and recombinant protein domains and modules.

One of the challenges in understanding the wealth of data from the human genome project is to understand how the 30,000 or so proteins interact and carry out their functions. There is a need for libraries of proteins and an initiative from the Dunn School by A Neil Barclay and Marion H Brown has established the Oxford Module Consortium with the help of groups from the Dunn School and also the Weatherall Institute for Molecular Medicine, the Wellcome Trust Genetics Centre of Human Genetics, the Biochemistry Department and the Physiology, Anatomy and Genetics department. This has been possible thanks to initial funding from the John Fell Oxford University Press Research Fund. The OMC will provide libraries of reagents – both DNA constructs and purified recombinant protein to researchers. It will concentrate on domains or modules – the parts of proteins that can fold independently. The OMC is interacting with groups worldwide to exchange reagents and make these resources widely available.

More details available at www.omc.ox.ac.uk

Obituaries

Laurence Turley

19th Jan 1950 – 4th Nov 2006

We are sad to report the untimely death of Laurence who worked for almost half his life for Siamon Gordon in the Dunn School.

Laurence was one of five siblings brought up in Old Headington. He himself had three children and two grandchildren. He was immensely proud of his family.

Laurence was one of the old school of senior technical staff who provide general support for all those in the lab. He helped to launch many of the DPhil students who have since become famous in their own right. In addition to macrophage and animal experiments he looked after the IT for the lab and repaired much apparatus.

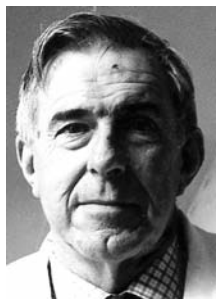
Outside his work Laurence was a keen participant and spectator of many sports. He captained the Dunn School cricket team to at least one 'championship' and was a keen squash player. He followed Oxford United through the good times and the bad. He was also an avid listener to a wide range of music.

During the two years since the diagnosis of his colon cancer Laurence was characteristically brave. While opting for the most intensive treatment he nevertheless tried to protect his family from the real truth of his outlook.

At his memorial service Derralyn Hughes spoke of the "charming, cultured, sensitive, diffident and loving man we knew". He will be sorely missed by many.

John Tobin

Died on Feb 5th 2007. He was 88.



John was one of a long line of distinguished microbiologists who took on the role of Departmental Demonstrator after their official professional retirement. He was in the Dunn School from 1980 to 1985.

John qualified BM, BCh, in Oxford in 1942, took the Dip. Bact in 1948 in Manchester where he worked for many years. He was honoured with the FRCPath in 1970 and the FRCP in 1979 and finally he took the DM in 1991.

He retired from the Directorship of the Public Health Laboratory in Oxford in 1980 and transferred to the Dunn School where he was actively involved in the early studies on the classification and epidemiology of Legionella pneumophila. He published at least a dozen papers during this time.

John will be remembered by those that knew him as a modest, kind and thoughtful man who was always ready to help young scientists. He was also a witty, cheerful and entertaining companion.

Mike Puklavec

26 Dec 1952 – 9 July 2007

Just as we prepared to go to press we were sad to learn of the sudden, unexpected and untimely death of Mike Puklavec on 9th July 2007 aged only 54.

Tributes have been flowing in from several generations of students, visitors and senior staff who all benefited from his technical skills, scientific advice and unfailingly cheerful companionship.

MikeP as everyone knew him, arrived at the Dunn School in 1979, working first with Mike Bramwell and Professor Henry Harris, and then moving in 1980 to take responsibility for tissue culture and the preparation of monoclonal antibodies in the MRC Cellular Immunology Unit under Alan Williams. For the last 27 years Mike has been responsible for the preparation and husbandry of all monoclonals from about OX25 to OX130, a huge contribution to immunological research not only in Oxford but around the world. He was dedicated to, and passionate about his work, a real professional who thought a good deal about the research flowing from the application of the OX monoclonals.

On the personal front everyone who worked with him speaks of his kindness, thoughtfulness and sense of humour. Mike was a lifelong batchelor but was very close to his sister and her children. In Bicester he played a major role in the community and the Methodist church.

History Corner

Ritchie was clearly on the ball and students starting immunological research today might be well advised to start with Ritchie's 105 year-old review!

100 years ago: James Ritchie leaves, Georges Dreyer appointed first full Professor. It is perhaps appropriate to note in this 'Immunologically-themed edition of *Fusion* that James Ritchie, who was the first Lecturer in Pathology appointed by the University in 1897 wrote several immunological papers.

His major contribution was a review of the then current theories of immunity presented first as a DM thesis at Edinburgh University (where it was awarded a gold medal) and then published in three 'episodes' in the Journal of Hygiene, 1902, 215-50, 251-285 & 452-464 entitled "A Review of Current Theories Regarding Immunity". Some parts of the papers have a surprisingly contemporary feel to them, "cholera in man is almost certainly a toxic disease since the bacteria are confined to the intestine"-the difference between exotoxic and endotoxic diseases is clearly stated. "The word receptor is much more fitting to express the group within the cells which may carry an affinity capable of saturation by a molecule outside the cell". But in other places the terminology clearly shows its age with much talk about protoplasm. The papers conclude with 106 references starting with Metchnikoff 1896 and ending with Ehrlich 1901. These two scientists were to share the Nobel prize in 1906.

Ritchie was clearly on the ball and students starting immunological research today might be well advised to start with Ritchie's 105 year-old review!

Another Immunological landmark in the Dunn School history was the foundation in 1963 of the MRC Cellular Immunology Unit in the new building under the Directorship of Jim Gowans.

Reminiscences of the Dunn School

Celia Bungay (née Hammersley)

I was delighted to be invited by Paul Fairchild to revisit the Dunn School again 43 years after I had left to start our family. Graduating in Pathology from Cambridge in 1958 I was hoping to find a Virology post in Oxford as my fiancé, Geof, was coming to do his clinical medicine course at the Radcliffe Infirmary. It was normal practice for the Dunn School junior teaching post for the graduate medics to be filled by a Rhodes Scholar but, fortunately for me, there was no suitable applicant that year and I was duly appointed as Departmental Demonstrator in Pathology. The position offered the opportunity to lecture, help run the practical laboratory sessions and pursue research.

I was warmly welcomed on my first day by Dr Gareth Gladstone's technician, Jimmy Smith, a very keen weightlifter, as Dr Gladstone was on his annual holiday. I discovered that my lab was in the same area as those of many of the "penicillin team'' who then were working on cephalosporin C. Professor Sir Howard Florey, who was terrified of suffering from anaphylactic shock should he have a flu jab from his GP, refused to have the injection when it was offered but somehow managed to persuade my medical student fiance to go to his lab annually, armed with the necessary antedotes, and administer the vaccine to him and then remain with him for some considerable time to make sure there was no adverse reaction.

Fortunately for us there never was any problem. Another of the penicillin team, Dr Norman Heatley, was one of the kindest, quietest and unassuming people we ever met. He and his wife soon invited these newcomers from Cambridge to their home and made us feel so welcome. Dr Heatley's "Heath Robinson'' creations designed for the production of penicillin are world famous but just one illustration of his many outstanding abilities to solve practical problems, often on a minute scale.

Jimmy showed me all the ropes and warned me to be ready on Dr Gladstone's first morning for the daily routine on his arrival – he would take off his jacket, swing his arm around several

times, prick his incredibly enlarged thumb (from repeated usage) and allow a drop of blood to fall onto each of 100 immaculately cleaned glass cover slips. After the blood had clotted the clots were washed off and Staphylococcal leucocidin assays done on the leucocytes which had remained stuck to the glass.

I was involved in Dr Gladstone's staphylococcal toxin studies, working on hyaluronidase. Hyaluronic acid was too expensive to buy so, once a week, I cycled round to the Maternity Department in Walton Street to collect a large sweet jar full of umbilical cords which the midwives had put into acetone for me. The extraction of the acid involved handling the cords and I still get small cracks on the tops of my thumbs in winter which were believed to be a vestige of working with acetone. Health and Safety didn't have quite the same impact half a century ago!

After two years, I was able to realise my ambition of working on viruses as Dr John Watkins was appointed as University Demonstrator in Virology. I was to work on *Herpes simplex* virus which required HeLa cells in which to grow. For the CCY growth medium, I needed fresh calf serum so, once again, I embarked on a weekly cycle ride, this time to a slaughterhouse in Abingdon Road. My other regular requirement was a large supply of fertile hens' eggs but these had to be collected every Monday lunchtime by car from a farm in Garsington. My D.Phil. studies showed how, in patients, *Herpes simplex* virus could spread to uninfected cells in spite of the presence of circulating antibody. Time lapse cinematography showed that infected cells fused with neighbouring normal cells to produce multinucleate giant cells, and this occurred before any new virus was produced.

The other part of my work involved lecturing to the medics and demonstrating in all the practical classes. I felt as though I had been thrown in at the deep end – my first lecture as an inexperienced 21 year old female, to over 70 (mostly male) 22-24 year old students, was on *Neisseria*. Dr Gladstone had, at the beginning, given me some very sound advice which I have followed during my university and, more recently, sixth form teaching career – students

Celia Bungay

will try to catch you out by asking tricky questions but "no-one knows everything; don't try to make up answers – say you don't know but will find out and tell them next time." I soon realized that this strategy earned me enormous respect.

There was one great horticultural advantage of working at the Dunn School in those days. Every week during my last year, we took home two sacks of guinea pig manure from the animal house, thanks to Mr Kent. We dug it into our "building site'' garden and neighbours used to wonder why their broad beans were 18 inches tall while ours were over 3 feet!

Being shown around the department again, it was great to see how well much of the old building has been incorporated into the new development, to appreciate the vast increases in work and staffing which have occurred and to see all the modern equipment installed. I was certainly reminded how simple things were in my time: Sir Paul Fildes refused to have a phone in his lab as he didn't see why anyone should be able to demand his attention instantly or "jump the queue" to discuss matters by ringing rather than visiting him in person and waiting until he was free. I look back with much pleasure on my time at the Dunn School.

Interview with Oreste Acuto

I was interviewed by several group leaders, but I was seduced by the science and the people of the Immuno-biology Unit

Tell us a little about your background and what led you into science as a career?
I grew up in Latina, a town near the coast of the Tyrrhenian sea, 40 miles south of Rome, where my parents settled after World War II. The last one of four children, I attended primary and secondary public schools in my hometown, finishing with a scientific diploma in 1968. Latina was a new and quiet town established in the early 1930s, right in the middle of a vast reclaimed swamp, known as the ancient Pontin swamps. The region had been infested by malaria for thousands of years. However, at the time I was born, DDT had helped to get rid of the disease and the countryside looked very pleasant with a temperate climate all year-round and a prosperous agriculture, an idyllic landscape that my parents used to call "our little California".

Although my parents had planned that I would study economics (my father worked for a major Italian bank) and that I would get a "good job" in finance, by the time I finished high school, I had decided that I wanted to be a research scientist in biology. My "strong" argument that I had a passion for "understanding how molecules made up living organisms" together with the support of a family friend, a medical doctor, scientist and Professor at the University of Siena, convinced my parents that my "faith" would one day help me find a job that I liked.

At 18, I moved to Rome to study Biology at the major public university "La Sapienza" . Those years were quite turbulent times in Italian society. The Italian universities and Rome were centres of strong political fervour and of heated confrontation. Like many young Italians at that time, I was attracted by this intense social and political turmoil and actively participated in it, with the hope of contributing to important changes. Nevertheless, I still managed to accomplish my undergraduate exams ahead of time.

Where did you develop your interest in immunology and how has your career progressed since then?
When it was time for me to look for a laboratory where I could carry out experimental research work towards my doctorate, I had just heard of a new research institute in Rome (The Institute of Cell Biology of the Italian National Council of Research), led by the 1986 Nobel price winner, Rita Levi-Montalcini. I was interviewed by several group leaders, but I was seduced by the science and the people of the Immunobiology Unit directed, at that time, by Professor Franco Celada, who accepted me for the thesis work. Franco and Dr. Roberto Tosi, one of his assistants with whom I had to work, were so inspiring personalities and excellent teachers that I embraced immunology with enthusiasm, in spite of, I have to admit, my very poor understanding of it (I had taken only one exam in immunogenetics). Because of its complexity, immunology appeared to me scary but at the same time attractive (the former is still today the most common reaction of non-immunologists). The subject of my experimental work was to find where within the kappa chain of rabbit immunoglobulin, genetic markers (called allotypes) were distributed. My findings unequivocally demonstrated that one allotypic marker of rabbit immunoglobulin kappa light chain was located within the variable region. Considered emigmatic at that time, this result, published in 1975 in my first paper in the Journal of Immunology, could only be fully explained 15 years later. This taught me the need for perseverance and that being a scientist also means believing in your own work.

After my doctoral degree, I decided, "to take a short break" from immunology. I have always had a bent for explaining biological phenomena in molecular terms and I felt that my bio-molecular background was rather precarious. Of various options, I chose to spend two years in the laboratory of Professor Giorgio Semenza in the Biochemistry Department of the ETH in Zurich, where advanced research was carried out on biological membrane structure and function.

At the ETH, I was embebbed within an environment of excellent biochemists and biophysicists, expert in membrane lipids and proteins. It is this experience which laid the foundations for my knowledge in membrane receptor structure and function.

Of which of your many achievements in science are you most proud?

I then returned to immunology at the Swiss Experimental Cancer Institute (ISREC) in Lausanne, to spend three years as a post-doctoral fellow with Dr. Markus Nabholz trying to define membrane components involved in cytotoxic T cell-mediated lysis, including the much sought after T cell antigen receptor (TCR). However, my "rendezvous" with this field had to wait a little longer. At the end of 1981, I obtained a position as a Lecturer in Pathology to work in the Division of Tumour Immunology at the Dana Farber Cancer Institute, at Harvard Medical School. Once again, my task was to chase the then "mythical" TCR. This time, I was lucky. Indeed, my work contributed to securing the belief that the "ghost" that had been chased for so many years was now firmly in our hands. My work provided the first molecular evidence that the new receptor was responsible for antigen recognition and that, similar to the immunoglobulin, its two subunits were composed of both variable and constant domains. I then demonstrated that both subunits bore homology to immunoglobulin. The cloning of genes coding for the TCR gave me an opportunity, now with a group of my own, to decipher specific and key features of the TCR recognition of antigen and MHC. Although I recognise that I was very lucky to be in the right place at the right time, I am obviously particularly proud of the work I accomplished in Boston.

Tell us a little about your current research: what questions in immunology do you hope to address?

After this extraordinary experience at Harvard, I came back to Europe in 1988 to settle in Paris at the Department of Immunology in the Pasteur Institute. During the eighteen years I spent there, I developed a strong interest in understanding the molecular basis of T cell activation which is still the focus of the scientific activity I have brought with me to the Dunn School. During their entire life, T cells are controlled by a complex network of external cues that determine their fate and consequently the outcome of immune responses. We know now that the TCR, with its amazing capacity to decode and process incoming signals occupies a central position in this decision-making process. We also know that the origin and/or severity of many immunological dysfunctions, such as autoimmunity and allergy, often reside in the alteration of genes that control T cell signalling. It is, therefore, of central importance in modern clinical immunology to understand how the T cell signalling machinery is composed and how it reacts to set effective but safe conditions in an immune response. Our goal is to understand the molecular basis of TCR triggering and how it is processed by a complex multi-component machinery to orchestrate programmes of gene expression. We employ biochemical and genetic approaches using in vitro and in vivo experimental models to dissect and unravel how T cell signalling works. We hope that our work will contribute to a better understanding of immunopathologies and provide potential pharmacological targets to control them.

How have you found the transition to the Dunn School and to life in Oxford?

Moving to Oxford from Paris has been a great change both for my family (we have a 16 year old daughter) and for me, but we all like it very much. My daughter loves the European School in Culham where she attends a french Lycee. Transition has been quite smooth and pleasant, and we have discovered many new interests in Oxford, away from the big city. Yes, at times we dream about a real french baguette and a few other french delicacies..... but working at the Dunn School is simply great.

Which aspects of British culture do you like most and which would you rather forget?

The sense of humor, fairness and tolerance. And thus far, I have no complaints...

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