fusion

THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

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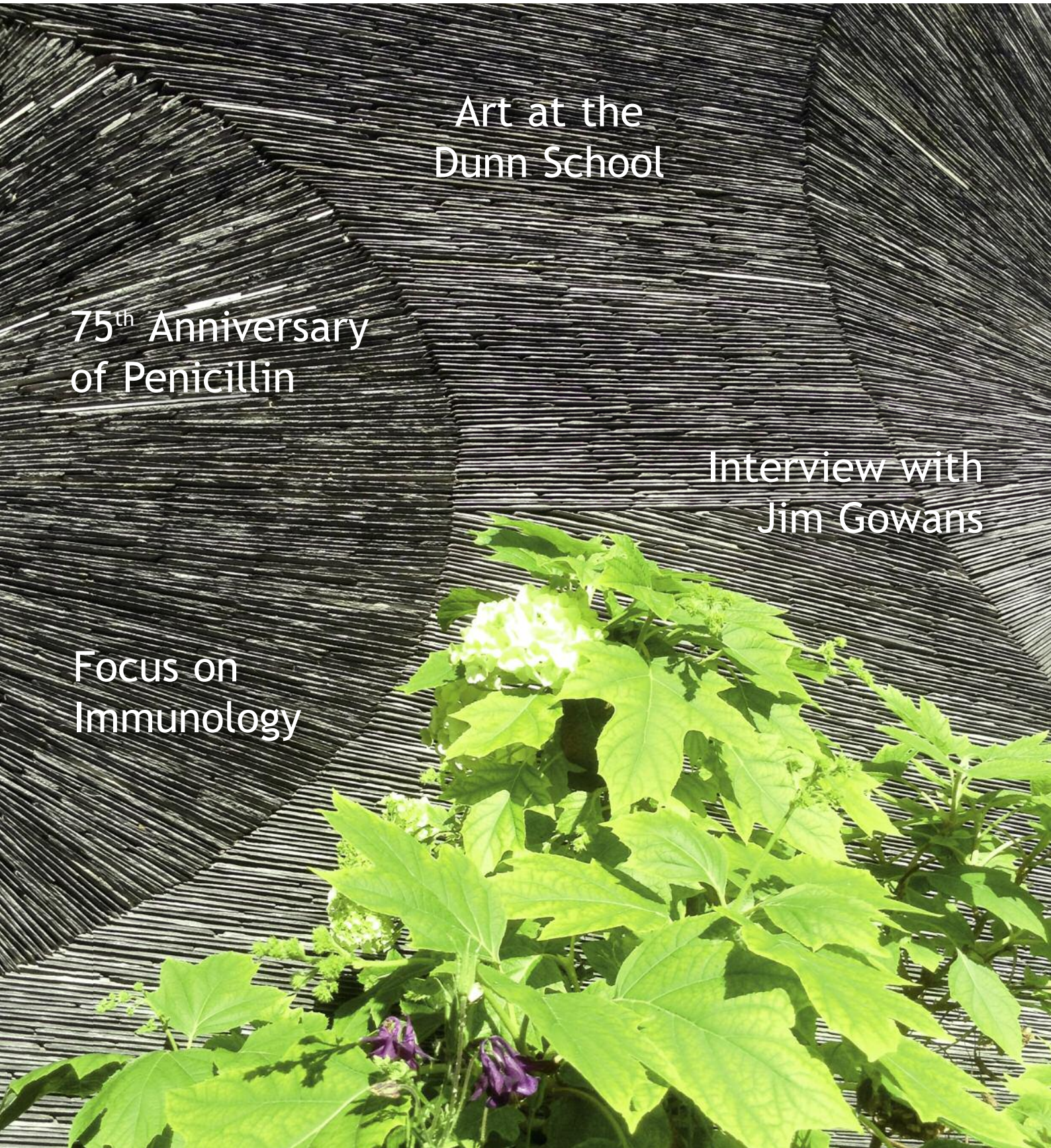
UNIVERSITY OF OXFORD



Art at the Dunn School

75th Anniversary of Penicillin

Interview with Jim Gowans

Focus on Immunology

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

Editorial

2016 is a special year for the Dunn School. More precisely, February 12th was a special day: it marked the 75th anniversary of the first therapeutic use of penicillin in humans. The story is well known: PC Albert Alexander, an Abingdon policeman, was treated in the Radcliffe Infirmary for a severe and life-threatening infection that may originally have come from a scratch from a rose thorn (sources vary on this). The response was dramatic: within hours Alexander showed signs of recovery. Unfortunately, there was not enough penicillin to maintain the dose for a full course, despite the Dunn School team recycling it from his urine, and he died a month later from overwhelming septicaemia. A sad story, but nevertheless a successful demonstration of the efficacy of penicillin. Although these dates are always a little artificial, this can legitimately be seen as the birth date of the antibiotic era. What is not at all artificial is that this revolution overcame one of the major causes of death across all human history.

Unsurprisingly, penicillin was voted in a BBC poll to be the greatest medical advance of the 20th century. Of course the story is more complex (and if you haven't read Eric Lax's outstanding book, *The Mould in Dr Florey's Coat*, I can highly recommend it), not least because of the alarming emergence of bacteria resistant to all antibiotics. The Dunn School's Chris Tang featured in an excellent discussion of the whole story on BBC Radio 4's, *In Our Time*, hosted by Melvyn Bragg. It is available on the BBC iPlayer if you missed it.

We are rightly proud of the Dunn School's world-changing discovery, and we have marked it in various ways. Most strikingly, we are organising a greatly expanded version of what is already our major annual event: the Norman Heatley Lecture, which honours a key member of the penicillin team ("…without Heatley, no penicillin", as Henry Harris famously wrote). This year's Heatley Lecture will be held in the spectacularly refurbished Weston Library of the Bodleian, and will be given by Jeremy Farrar, Director of the Wellcome Trust, the UK's largest charity. We would be very happy to see Dunn School friends and alumni at the lecture, so please let us know if you would be interested in attending.

Despite our pride in our past, our main efforts look to the future. I'm pleased to report that the Department is thriving, and that we continue to recruit new groups. Supporting young group leaders is the most satisfying aspect of my job as Head of Department, even more so when they are all doing so well. One measure of our ongoing success is the 42% increase in grant income over the last 3 years; a more important one is the quality and range of research that we are publishing. Sadly, the financial climate is harsh, and our research success does not directly translate into budgetary ease. We are, therefore, more grateful than ever to our benefactors: we are tremendously lucky to have friends who have supported the Department in many ways. A recent example has been that, upon his own retirement, Neil Barclay was instrumental in funding the endowment of a new professorship – the Barclay-Williams Chair of Molecular Immunology. This generous gift, from funds generated from royalties from monoclonal antibodies that Neil developed, and which has catalysed an additional gift from the EPA Research Fund, ensures that we can now recruit a new senior immunology group. The endowment provides support for that new professor in perpetuity. We are very grateful.

Let me finish by repeating a message from last year. We are lucky to have a great network of Dunn School supporters and alumni. This extended family is something that we greatly appreciate, especially in these uncertain times, when we value more than ever our fully European and international outlook. Please drop in and see us if you happen to be in town, and keep us up to date with your news; also keep an eye on our website, where we regularly report new developments. Most importantly, however, we do hope you will enjoy reading this edition of *Fusion*, which gives you further insight into the life of the Dunn School and our ongoing research.

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Front cover image:
The Molecular Pools, built of slate and designed by Heather Ackroyd and Dan Harvey. Photograph by Paul Fairchild.

News

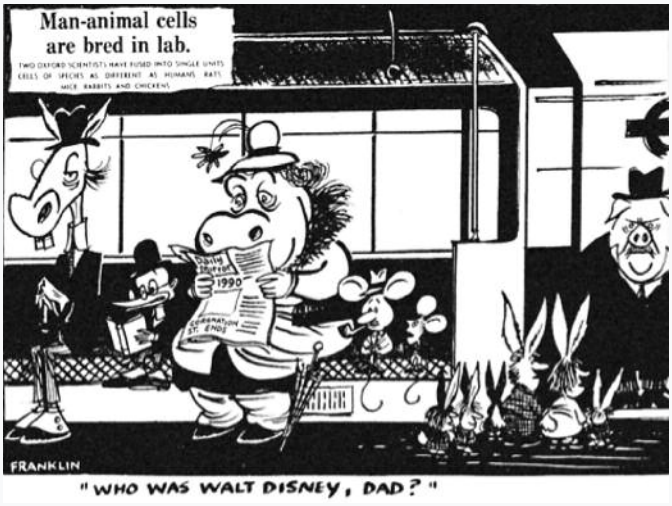
The BBC's *One Show* Pays Tribute to Norman Heatley

On 10th February 2016 the BBC's *One Show* broadcast an item about Norman Heatley to coincide with the 75th anniversary, two days later, of the first systemic administration of penicillin to a patient, the story of Albert Alexander being described later in this issue. The piece paid tribute to the pioneering work of 'the Oxford team' headed by Howard Florey and featured footage from the Dunn School, filmed during the early days of penicillin. However, the piece focussed primarily on the frequently overlooked role played by Norman Heatley in the purification of penicillin in its active form, a story that lent itself well to the series commissioned by the BBC on the theme of 'unsung heroes'. The film was narrated by Michael Mosley and featured an interview with Eric Sidebottom from the Dunn School and included the recollections of Norman Heatley's daughter, Rose. The panel shows images taken from the film.

Belated Anniversary of the First 'Cell Fusion' Paper

Last year saw the 50th anniversary of the publication of the paper that gave rise to the title of this magazine. On 13th February 1965, Henry Harris and John Watkins published their ground-breaking paper entitled *"Hybrid cells derived from mouse and man: artificial heterokaryons of mammalian cells from different species"*.

As Harris reported in his 1970 book, *Cell Fusion*, reviewing the early research resulting from this technique "the newspapers of the world were not slow to appreciate the biological significance of this discovery". The best remembered is the cartoon from *The Daily Mirror* that appeared on the 15th February.



One reaction to the discovery that cells from different animal species could be fused together to form viable hybrids. Reproduced with kind permission of the Daily Mirror.

The cell fusion technique has now been applied in many fields of biological research and has made major contributions to the development of monoclonal antibodies, gene mapping and the analysis of malignancy, including the discovery of tumour suppressor genes.

Alemtuzumab Used in Pioneering Treatment for Leukaemia

Towards the end of last year, Great Ormond Street Hospital reported the revolutionary treatment of a one year old girl for drug resistant acute lymphoblastic leukaemia (ALL). The ground-breaking approach made use of various technologies, one of which originated from work at the Dunn School conducted by Herman Waldmann and his group.

The therapy constituted an off-the-shelf cellular treatment, using genetically-modified T cells to ablate the recipient's leukaemia. The T cells were armed with a chimeric receptor composed partly of an antibody specific for a surface antigen (CD19) expressed by all B cells (including leukaemic B cells) and partly of the intracellular components of the T cell receptor capable of activating the T cells to deliver a lethal hit to the tumour cells. As the off-the-shelf T cells are grown from one individual they would normally be rejected by the recipient. In this pioneering treatment, however, rejection was prevented by using Alemtuzumab, a monoclonal antibody specific for CD52 developed by Herman Waldmann and his colleagues and recently approved by NICE for use in the treatment of multiple sclerosis (reported in *Fusion* 13). In the present case, however, Alemtuzumab was used to severely deplete the host T cells that would otherwise have rejected the therapeutic inoculum.

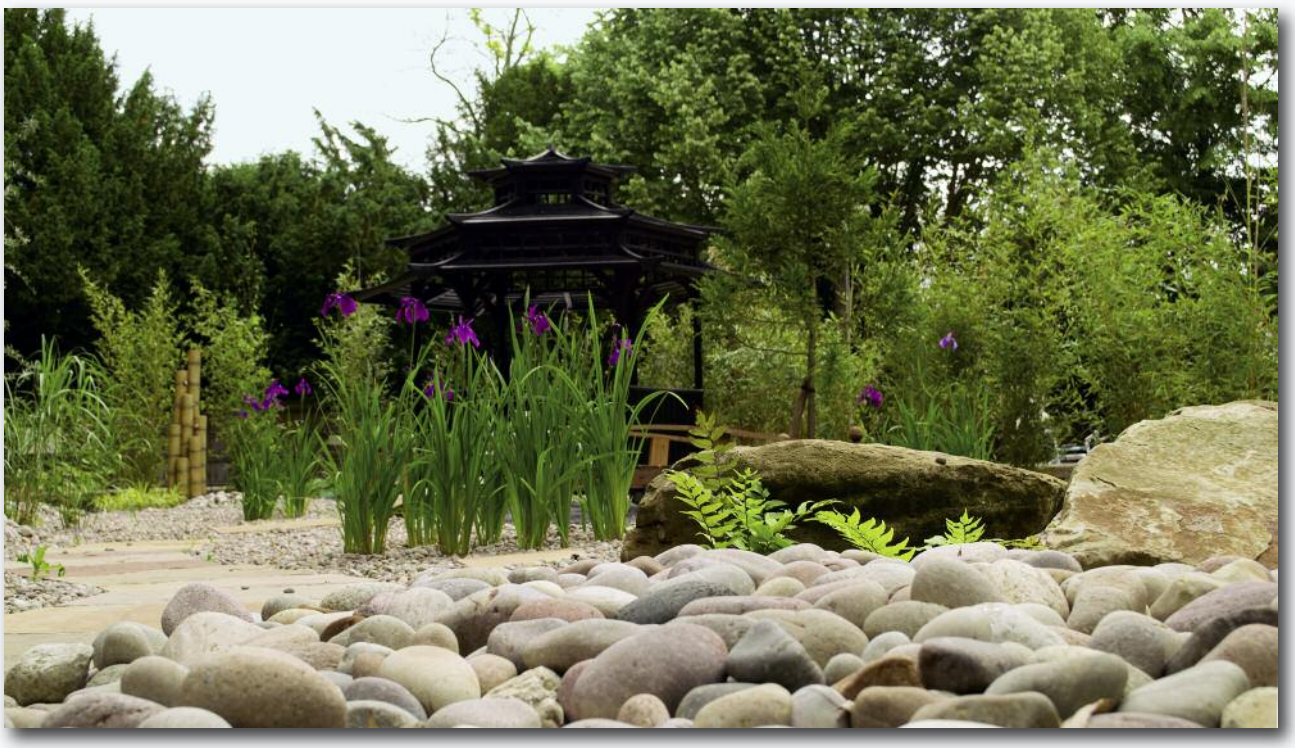
So that the off-the-shelf inoculum was not touched by Alemtuzumab, the CD52 gene in the donor T cells was excised. This provided them with a therapeutic 'window' in which they could attack the malignancy without risk of being targeted by the Alemtuzumab. The attraction of this pioneering treatment is that it does not have to be personalised to the T cells of each individual patient, thereby enabling good quality control of a uniform source of cells. This case suggests that off-the-shelf T cells bearing chimeric receptors for distinct malignancies may be available for cancer therapy in due course and that their use may be facilitated by conditioning of recipients with a drug that has its origins at the Dunn School.

Elizabeth Robertson Receives the Royal Society's Royal Medal

Congratulations to Elizabeth Robertson FRS who was awarded the 2016 Royal Medal for 'her innovative work within the field of mouse embryology and development, establishing the pathways involved in early body planning of the mammalian embryo'. She joins the list of distinguished Dunn School faculty members previously honoured as Royal Medalists including Richard Gardner (2001), Henry Harris (1980), EP Abraham (1973), and Howard Florey (1951). The Royal Medals, also known as the Queen's Medals, originally created by King George IV in 1825 are awarded annually by the Sovereign for outstanding scientific achievements contributing to the advancement of knowledge.

Liz is well known for her early work establishing the feasibility of embryonic stem cell technology for engineering defined mutations into the germ line and pioneering gene targeting strategies for generation of knock-out and knock-in reporter alleles. She was especially pleased to be recognised for her genetic dissection of the signalling pathways controlled by the secreted growth factor Nodal and its downstream Smad2 effector at early post-implantation stages during establishment of the anterior-posterior body axis, positioning neural and mesodermal tissue progenitors on opposite sides of the embryo. An especially important contribution was the discovery that Nodal/Smad2 signals are required within the extra-embryonic endodermal cell population overlying the embryo proper, previously viewed strictly as a supporting tissue. Her analysis of the Smad2 mutant phenotype revealed for the first time that signals arising from a discrete endodermal subpopulation located on the anterior side of the embryo, the so-called Anterior Visceral Endoderm, ensure primitive streak formation exclusively on the posterior side of the embryo and thus anterior-posterior identity.

Her work has exploited mouse genetics to investigate cell fate decisions in the developing embryo. In a seminal paper in *Nature* she used a knock-in strategy to generate a Nodal LacZ reporter allele. The increased sensitivity afforded by LacZ staining unexpectedly revealed a novel domain of nodal expression on the left side of the embryo before the major organs including the heart, lungs, and gut become asymmetrically positioned within the body cavity. She was able to demonstrate that the Nodal signalling pathway is responsible for establishment of the left-right body axis. Subsequent work by other labs studying development of numerous model organisms including fish, frogs, chicks, even snails, has shown that this genetic cascade is tightly conserved across evolution.



Photograph courtesy of Tim Davies

Art and Pathology: Unlikely Bedfellows

The Sir William Dunn School of Pathology has a long history of appreciation of the art world and has commissioned various pieces of art that may be viewed throughout the Department. These include the photographic works of Catherine Yass which adorn the walls of the Combination Room and which were the culmination of a year-long residency in Oxford as part of the millennium celebrations for the Year of the Artist.

The Molecular Pools, built of slate and designed by Heather Ackroyd and Dan Harvey, were originally located in the small garden adjacent to the Leslie Martin Building but were dismantled during the construction of the new Oxford Molecular Pathology Institute (OMPI) building. They have, however, since been resurrected in their existing form, consisting of vertical panels on the east-facing wall of the OMPI, featured on the front cover of this issue of *Fusion*.

In recent years the appreciation of art has developed into a collaborative venture with the Ruskin School of Drawing and Fine Art through the award of an annual art prize. In 2013, Vienna-based artist, Lara Verena Bellenghi, a graduate of the Ruskin School, won the prize for her work reflecting the 'ebbs and flows of the bodily interior', a composite piece of numerous transparencies, one of which is shown in Figure 1. The work was highly commended by the awards committee for its ability to communicate the breadth and complexity of the research environment at the Dunn School.

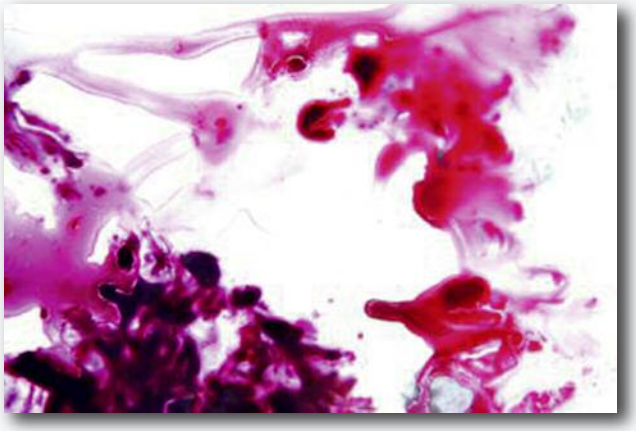


Figure 1.

Julia Sklar (St Hugh's) won the 2014 prize, which she shared with Emma Papworth (Queens College), for her steel wire depiction of a human face in three pieces that progress from the abstract to the concrete. The piece can currently be viewed, as intended, from below in the Combination Room (Figure 2). Describing her work, Julia said: 'When read from top to bottom, the forms grow increasingly concrete. This evokes the manner in which the School's pathologists condense complex information into a lucid history'.

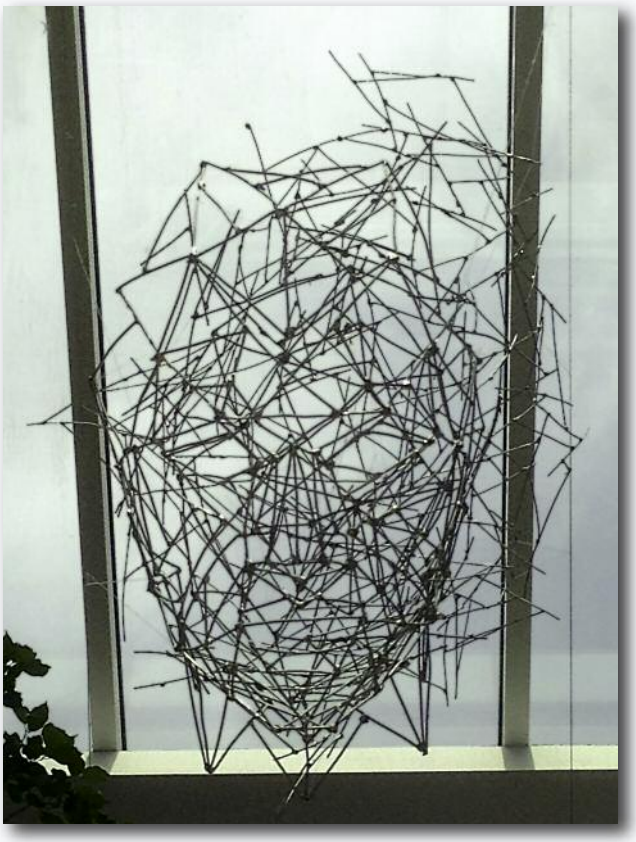


Figure 2.

The most recent awards, have featured the works of Eleanor Minney and Mariette Moore whose intricate pencil drawing (Figure 3) was inspired by images of the heart, stomach and small intestine. The work includes fanciful images based on cellular structures from which Moor claims 'a mass of one hundred and thirty six beings evolved and formed a landscape'.

Figure 3.

RESEARCH FEATURE

Exploring Mechanisms of Genome Stability

Christopher Carnie and Dragana Ahel

Our laboratory focuses on the family of proteins called SNF2 ATPases, and their role in the maintenance of genome stability. SNF2 ATPases are functionally diverse and perform a range of DNA- and chromatin-associated functions, with a number already established as DNA damage response (DDR) factors. Many SNF2 ATPases, however, are still poorly-characterised. Our goal is to investigate some of these proteins, as we believe that advances in this field could have a significant impact on our understanding of the molecular mechanisms of human disease and cancer.

The DDR is a highly complex network of interconnected pathways, which have evolved to minimise the deleterious effects of a broad range of DNA lesions. Without these pathways, environmental and endogenous mutagens could cause extensive genomic instability. Indeed, deficiencies in DNA repair factors have been attributed to a large number of human diseases, including Fanconi anaemia, ataxia telangiectasia and Cockayne syndrome. Such deficiencies also extend to many cancers. For example, heritable mutations in the *BRCA1* and *BRCA2* genes greatly increase the risk factor for breast and ovarian cancers.

In addition to the pathological implications surrounding defects in the DDR, its pathways are also of great relevance to many cancer treatments currently used in the clinic. A range of these drugs – such as DNA crosslinking agents, topoisomerase inhibitors and PARP inhibitors – attack DNA and therefore pose extreme challenges to DNA replication in rapidly-dividing cancer cells. Furthermore, cancer cells may develop resistance to such drugs by altering the regulatory landscape of the DDR in order to accommodate a deficiency in a certain pathway. This highlights the interconnected, dynamic and redundancy-laden nature of the DDR. The DDR is, therefore, the subject of intense focus, in the hope that understanding this complex field will illuminate new therapeutic opportunities. There is close interplay between the DDR and the transcription and replication machineries and, while there is much yet to be understood, these touching points are under extensive investigation.

Classically, DDR pathways have been considered in relatively linear terms, with a specific set of factors functioning in the response to specific genotoxic insults. This approach has yielded many important findings and uncovered a number of important signalling hubs, but we may have failed to recognise crucial regulatory mechanisms governing the mode of resolution of DNA lesions in more complex or unusual cellular settings. We feel that the study of poorly-characterised SNF2 ATPases with implications in the DDR will yield important advances in our understanding of this complex field.

SNF2 ATPases

SNF2 ATPases are modular proteins characterised by a conserved catalytic core that supports DNA translocase function. In addition, they contain a breadth of diverse regulatory domains, which mediate context- and location-dependent activities of specific SNF2 ATPases (Figure 1). SNF2 ATPases function across a range of DNA- and chromatin-associated contexts, including nucleosome repositioning and chromatin remodelling, transcriptional regulation, DNA replication and DNA repair. They are found across all eukaryotes.

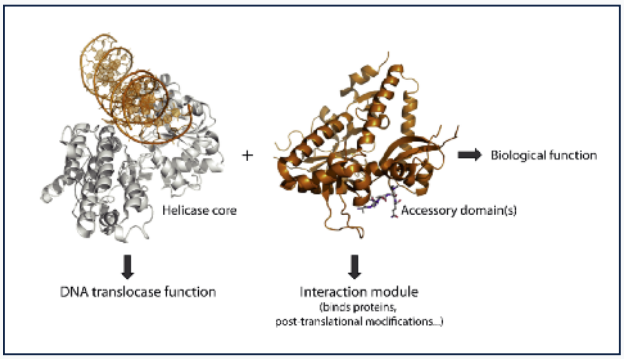


Figure 1. *The characteristic helicase core of SNF2 ATPases confers ATP hydrolysis-coupled DNA translocase activity. Accessory domains function as regulatory units mediating interaction with proteins, DNA and post-translational modifications. The specific combination of these domains defines biological function of a SNF2 ATPase.*

A number of SNF2 ATPases already have established roles in the DDR, but some relatively well-studied ones retain a degree of mystery. SNF2 ATPases with known roles in the DDR include – but are not limited to – ALC1, ZRANB3, CSB and Rad54.

The functional and regulatory elements found in a number of DDR-related SNF2 ATPases are still incompletely understood and present an attractive target for in-depth structure-function studies. Such studies offer mechanistic insights into the precise roles of these proteins in the DDR and enable us to better understand the processes in which they participate. ALC1 and ZRANB3 are two SNF2 ATPases in which we have deep interest, and in terms of approach, we aim to answer important questions using techniques ranging all the way from structural biology and biophysics to *in vivo* studies, making use of the excellent and broad research facilities within the Department and more broadly on South Parks Road.

SNF2 Spotlight: ALC1

We have previously identified ALC1 as a novel DNA repair factor with chromatin remodelling function (Figure 2). One of the challenges has been understanding how DNA repair machinery operates in eukaryotic cells, where DNA is embedded in chromatin. Our work has shown that ALC1 is recruited to sites of DNA damage by its interactions with a specific post-translational modification, poly(ADP-ribose). Poly(ADP-ribose) (PAR) is synthesized at sites of DNA damage by poly(ADP-ribose) polymerase 1 (PARP1) and our data show that PAR acts as a signal for the recruitment of DNA damage

response factors, including ALC1 [1, 2]. Recruitment of ALC1 to sites of DNA damage is strictly dependent on active PAR synthesis and is abrogated upon chemical inhibition of poly(ADP-ribosyl)ation by PARP inhibitors. Furthermore, recruitment of ALC1 is supported by its C-terminal Macro domain, which acts as a PAR-binding module (Figure 2). Therefore, ALC1 chromatin remodelling activity can be targeted to precise chromatin locations to support DNA repair.

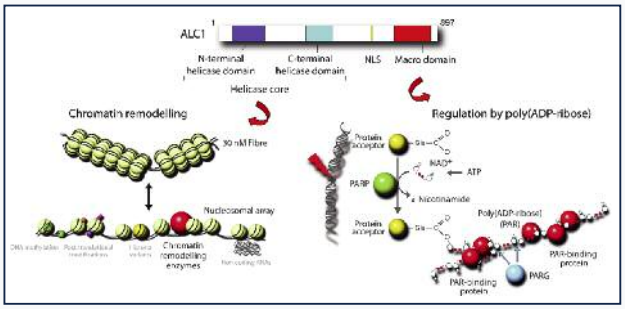


Figure 2. *Model of ALC1 function. Upon induction of DNA breaks, PARP1 catalyses formation of poly(ADP-ribose) (PAR) from NAD+. PAR acts as a platform for the recruitment of PAR-binding proteins, which contain PAR-binding domains. One such domain is the Macro domain, which recruits ALC1 to the sites of DNA damage. ALC1 can then perform chromatin remodelling function (supported by its helicase core domain) in order to relax chromatin and render it accessible to repair factors.*

Interestingly, overexpression of ALC1 is associated with cancer, indicating that its nuclear activity needs to be carefully regulated. ALC1 is frequently amplified and overexpressed in hepatocellular carcinoma (HCC) [3]. Furthermore, recent evidence suggests a direct role of ALC1 in cancer: its overexpression dramatically increased tumorigenicity in a xenograft mouse model, while its ubiquitous-expression in a transgenic mouse resulted in spontaneous tumour formation [4]. Moreover, overexpression of ALC1 promoted HCC progression and metastasis in mice, and seemed to correlate with poor prognosis [5]. However, despite these findings, the exact molecular mechanism by which ALC1 promotes tumorigenesis is not understood. Therefore, one of our goals is gain insight into how chromatin remodelling and poly(ADP-ribose)-dependent ALC1 functions contribute to its tumorigenic potential.

SNF2 Spotlight: ZRANB3

ZRANB3 (Zinc finger Ran-binding domain-containing protein 3) is a SNF2 ATPase involved in the replication stress response. Replication stress is one of the major sources of genome instability and occurs when a replication fork is slowed or stalled by damaged DNA [6]. It has been recognized as a factor contributing to the development of cancer [7, 8], and strategies to resolve replication blocks are, therefore, critical for the maintenance of genome stability. Cellular capacity to deal with replication stress relies upon multiple and redundant pathways [9]. The coordination between these pathways is mediated through PCNA (a DNA clamp which supports processivity of eukaryotic DNA polymerases), whose post-translational modifications (such as mono and polyubiquitination) play a major role in the selection of appropriate responses [10, 11].

Our data show that ZRANB3 plays a role in one of the pathways involved in the processing of replication blocking lesions in the leading strand DNA template [12]. ZRANB3 interacts with the key replication factor PCNA and subunits of the replicative helicase complex MCM. Furthermore, it localises at the sites of DNA replication and is recruited to the stressed replication forks by interactions with PCNA and polyubiquitin chains. It is also recruited to DNA breaks in a manner that resembles recruitment of other replication associated factors, such as PCNA and FEN1, but is mechanistically distinct from the recruitment of SNF2 ATPase ALC1. The roles of ZRANB3 in DNA replication and repair are supported by a number of structural domains, which provide specificity for a series of defined substrates, including PCNA, K63-polyubiquitin chains, and branched DNA structures (Figure 3). Most importantly, we have shown that ZRANB3 exhibits unusual structure-specific ATP-dependent endonuclease activity, which is contained within its C-terminal HNH domain [12]. However, biological relevance and specific aspects of ZRANB3 function are not fully understood, and are the subject of our research interest. Detailed understanding of the function played by ZRANB3 in the replication stress response would also allow us to study relationships between different pathways, known to play essential roles in the maintenance of genome stability.

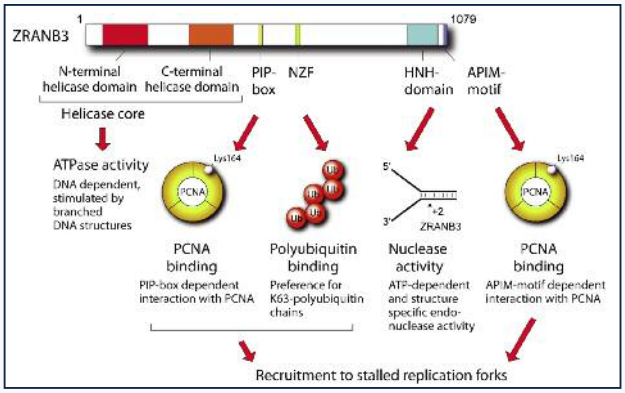


Figure 3. *Structural features of ZRANB3 relating to its biological function. ZRANB3's helicase core performs DNA-dependent ATPase activity, which is stimulated by branched DNA structures. ZRANB3 is recruited to stalled replication forks by two motifs, PIP-box and APIM-motif, which mediate interactions with PCNA. The NZF-motif supports this recruitment by providing additional interactions with polyubiquitin chains. The C-terminal HNH domain confers ATP-dependent, structure-specific endonuclease activity.*

Concluding Remarks

A large number of questions remain concerning the intricacies of the DDR and its complex interplay with the transcription and replication machineries. SNF2 ATPases have crucial roles across the spectrum of nuclear functions and it is our opinion that by utilising a broad range of techniques to study this diverse family of proteins, we may uncover new insights into the complex world of the DDR. With a deeper understanding of these crucial processes, we hope to uncover novel therapeutic strategies to combat diseases such as cancer.

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TECHNOLOGY FEATURE

New Microscopes for the Dunn School Bioimaging Facility

Errin Johnson and Alan Wainman

Since our last update in the 2013 edition of* Fusion*, microscopy at the Dunn School has continued to go from strength to strength. We have upgraded the Facility with new instrumentation, including the Olympus LiveCell confocal and the Gatan OneView CMOS TEM camera, and added a new staff member, Dr Anna Pielach, in the Electron Microscopy (EM) laboratory. As a result, we have a record number of users and are facilitating a diverse and exciting range of projects from both within the Dunn School (showcased in our inaugural Bioimaging Competition last year, see opposite page) and across the wider University. Here, we highlight two new additions to the Facility in 2016 that bring higher resolution imaging with both light and electron microscopy to our users.

Super-resolution microscopy

Super-resolution light microscopy has arrived at the Dunn School! Our new microscope, purchased in collaboration with Micron Oxford, consists of a Zeiss 880 confocal microscope fitted with an Airyscan, an array of concentrically-arranged hexagonal detectors. This technology presents significant advantages. In a traditional confocal system, light passes through a pinhole, resulting in partial loss of emitted light. In the Airyscan this additional light is collected, making it much more sensitive than a standard confocal (Figure 1). In addition, the array detector enables an increase in resolution 1.7 times beyond the resolution limit of traditional light microscopes ('Abbe's diffraction limit). This increase in both sensitivity and resolution makes our new microscope ideal for imaging objects which are very faint and/or too close together to resolve using a standard confocal – especially in living cells, as the Zeiss 880 is fitted with a 37 °C incubator and CO_2 control.

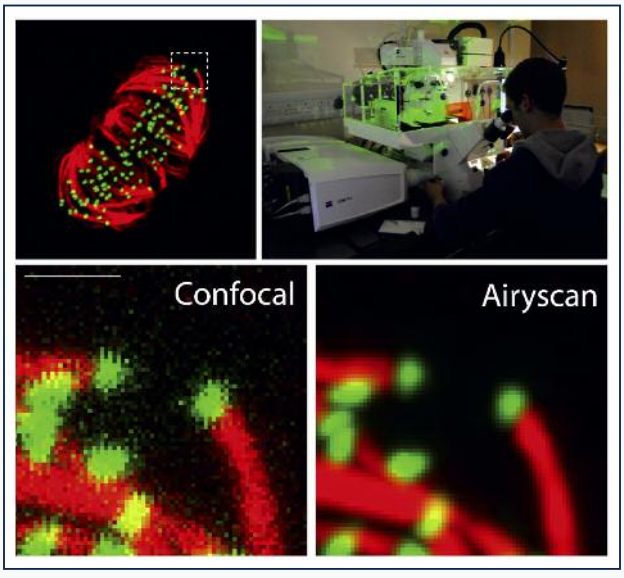


Figure 1. Comparison of confocal and Airyscan modes on the Zeiss 880. Images show kinetochore / microtubule attachment in HeLa S3 cells, with GFP-CENP-A as a kinetochore marker and microtubules labelled with anti-alpha-tubulin in red. Scale bar is 1μm. Courtesy of James Bancroft (Gruneberg Group).

Scanning electron microscopy (SEM)

We have recently installed a new Field Emission Gun (FEG)-SEM, the Zeiss Sigma 300, which was generously funded by the EP Abraham Cephalosporin and EPA Research Trust Funds. Our new FEG-SEM significantly upgrades our imaging capabilities, with 10-fold better structural resolution than the previous SEM. Thus, fine structures on cell surfaces, which we were hitherto unable to satisfactorily image, can now be beautifully resolved (Figure 2).

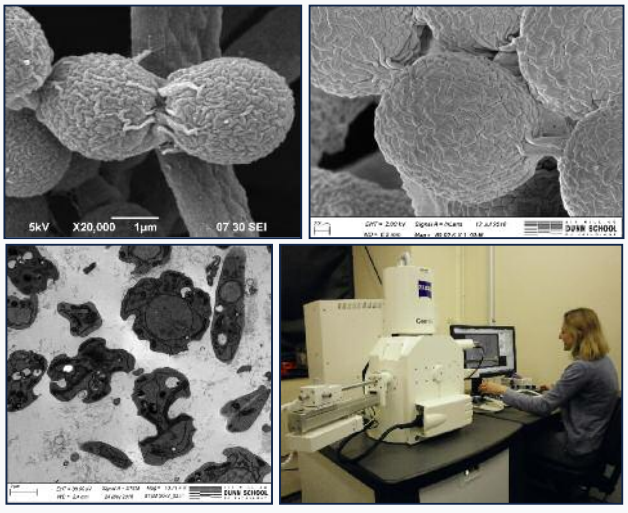
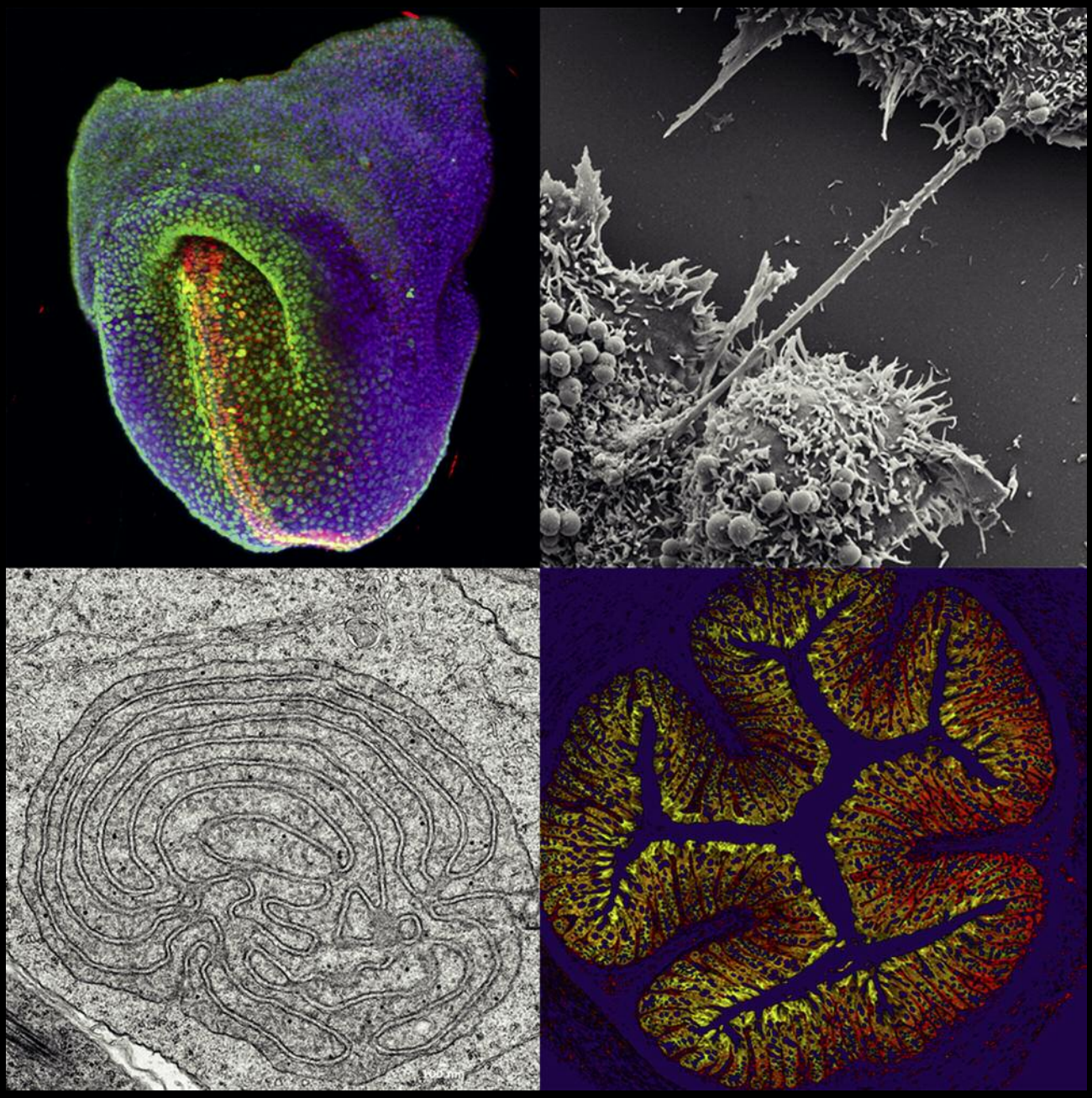


Figure 2. Top: Images of *Penicillium* spores images on our old SEM and our new Zeiss Sigma 300. Bottom: A TEM-like image from a resin section of Leishmania mexicana, imaged using the STEM detector on the Sigma 300. Anna Pielach enjoying using our FEG-SEM.

The Sigma 300 is easy to use and possesses multiple detectors for enhanced functionality:

- Two secondary electron detectors for low noise, higher resolution imaging
- A STEM detector for producing TEM-like images of ultrathin resin sections. Up to 12 grids can be loaded at once, making it ideal for high throughput screening of TEM samples
- A back scatter electron detector for elemental contrast, for imaging structures immunolabelled with colloidal gold particles, as well as for volume array tomography

Dunn School Bioimaging Facility Image Awards 2015



Top left: *Winner, Light Microscopy & Overall Winner: Mouse embryo (E7.75) stained for Foxa2 (green), Brachyury (Red) and DNA (blue) by Ita Costello (Robertson-Bikoff lab) on the FV1000 confocal.*

Top right: *Winner, Electron Microscopy —* Neisseria cinerea *binding to human bronchial epithelial A549 cells by Mirka Woermann, Rachel Exley (Tang lab) & Anna Pielach, Errin Johnson (EM Lab) on the Zeiss Merlin FEG-SEM.*

Bottom left: *Runner-up, Electron Microscopy — Fused mitochondria in* Drosophila *tissue by Saroj Saurya (Raff lab) on the Tecnai12 TEM*

Bottom right: *Runner-up, Light Microscopy — Mouse mid-colon stained for E-cadherin (yellow), IL18 (orange) and DNA (red) by Johanna Pott (Maloy lab) on the FV1000 confocal*

FOCUS ON IMMUNOLOGY

Immunology as a discipline has been integral to the Dunn School since the pioneering experiments conducted by Nobel Prize-winner, Sir Peter Medawar, the centenary of whose birth we reported in the last edition of* Fusion. *In this issue, our Focus on Immunology showcases the research of three different laboratories working to understand the mechanisms by which different facets of the immune system operate in health and disease.

Autophagy: Self-Eating your Way to a Healthy Gut

Agnieszka Kabat and Kevin Maloy

Whenever we become infected with a pathogen, our body responds by launching a series of protective immune reactions, however, unabated immune activation can cause chronic inflammation that damages host tissues. One example of this type of disease is inflammatory bowel disease (IBD), a chronic inflammatory disorder of the gastro-intestinal tract. Recent genetic screens have placed a process known as autophagy — a cellular degradation pathway — as a central player in IBD. However, the mechanistic basis through which mutations in autophagy genes predispose to IBD remains obscure. Our lab investigates how autophagy regulates immune responses in the intestine, in order to identify novel therapeutic approaches for IBD.

The gut represents a puzzle for our immune system: it must constantly monitor a vast surface (the area of a tennis court) for the presence of pathogens, whilst simultaneously tolerating trillions of beneficial commensal bacteria and food antigens. The precise mechanisms by which the intestinal immune system maintains this delicate balance are unclear, however its importance is underscored when the balance goes askew, leading to the development of chronic intestinal disorders like IBD. Clinically, IBD is divided into ulcerative colitis (UC) and Crohn's disease (CD), which together affect about 1 in every 400 people in the UK. Currently, there is no cure and IBD patients typically require long-term treatment with immune suppressive agents and, in many cases, surgical intervention. The pressing need for new therapeutic approaches is further emphasized by the rising prevalence of IBD in children, with pediatric CD having increased by 3-fold over the last three decades.

Three main factors are important in the development of IBD: the genetic background of the host; environmental factors, such as diet and composition of the intestinal commensal bacteria; and perturbations in the host immune system [1]. In recent years, genome wide association studies (GWAS) have identified many predisposing genetic mutations that occur more frequently in IBD patients than the general population. Although GWAS are a powerful tool to screen for new genes and pathways that might play a role in IBD, they do not explain how these pathways affect intestinal homeostasis or why people can still harbor predisposing mutations yet not develop IBD. The answer may lie in the interplay of a particular gene with the host immune system and the environment.

Degradation and recycling of cellular components is critical for all eukaryotic cells to maintain cellular homeostasis. Autophagy is an essential intracellular process during which damaged proteins and organelles are enclosed in a membrane vesicle, then degraded and recycled to fuel the cell. Autophagy becomes particularly important when nutrients are limited and a cell is starving. However, autophagy also contributes to certain immune responses, for example the killing of intracellular bacteria and viruses [2]. GWAS identified alterations in autophagy genes in IBD patients, which was something of a surprise, as it suggested additional roles for autophagy in controlling chronic inflammation. In particular, a single nucleotide polymorphism (SNP) in *ATG16L1*, a gene encoding an essential autophagy protein, is strongly associated with an increased risk of IBD [3]. Interestingly, this mutation leads to instability of the ATG16L1 protein during cellular stress; therefore cells that bear this mutation have decreased levels of autophagy [4].

How might reduced levels of autophagy lead to the intestinal inflammation that is observed in IBD? Several groups have attempted to tackle this question by looking more closely at the functional consequences of alterations in autophagy on both immune and tissue cells present in the gut. Several studies have examined the role of autophagy/Atg16L1 in intestinal epithelial cells or phagocytic cells. These suggested that Atg16L1 was important for the secretory function of specialised intestinal epithelial cells known as Paneth cells, which release anti-microbial peptides into the intestinal lumen to regulate bacterial growth [2]. In addition, Atg16L1 was demonstrated to be involved in intracellular bacterial handling and in the regulation of inflammatory IL-1β secretion by phagocytes [2] (Figure 1).

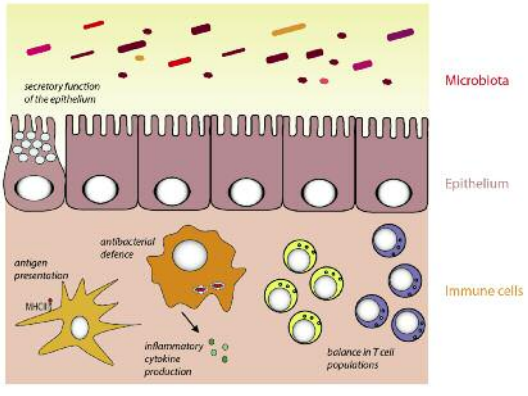


Figure 1. Autophagy regulates intestinal homeostasis through several mechanisms. Autophagy facilitates the secretory function of specialized intestinal epithelial cells, enhances anti-bacterial defence and regulates production of pro-inflammatory cytokines such as IL-1β by phagocytes, contributes to antigen presentation by dendritic cells, and regulates the balance of distinct $CD4^+$ T cell populations in the intestine.

Our group examined whether autophagy also regulates adaptive immune responses in the intestine. In particular, we assessed whether defects in autophagy affected intestinal $CD4^+$ T cells. $CD4^+$ T cells encompass several distinct subpopulations that are crucial for the induction and regulation of

adaptive immune responses in the gut and aberrations in their numbers and functions are often seen during intestinal inflammation [1].

We generated transgenic mice in which the autophagy gene *Atg16L1* was selectively knocked out in T cells. Although these mice initially developed normally, as they aged they displayed marked signs of intestinal inflammation and failed to gain weight at the same rate as age-matched control mice. Somewhat counter-intuitively, despite the intestinal inflammation, we saw reduced numbers of T cells in these transgenic mice. However, detailed examination of intestinal $CD4^+$ T cell subsets revealed that Atg16L1 deficiency differentially affected distinct subsets of $CD4^+$ T cells. A lack of autophagy strongly reduced the numbers of regulatory cells (Treg) in the gut, whereas the Th2 cell population was selectively expanded [5]. Why is this important? Treg cells play an essential role in maintaining a tolerogenic environment in the intestine; they are a counterbalance for effector T cells and keep pro-inflammatory responses in check. Indeed, a lack of Treg cells in humans leads to development of a fatal, multi-organ, inflammatory disorder, termed IPEX syndrome, which includes intestinal inflammation. On the other hand, Th2 cells are the key effector T cells that co-ordinate protective immune responses against intestinal parasites, but they are also responsible for driving harmful hypersensitivity diseases, such as food allergies and asthma. In line with their enhanced intestinal Th2 responses we found that mice with T cell-specific deletion of *Atg16L1* had very high levels of antibodies towards dietary proteins and commensal bacterial antigens, indicative of hypersensitivity against antigens encountered in the gut. Thus, a lack of autophagy simultaneously reduces anti-inflammatory Treg cells and increases pro-inflammatory Th2 effector cells in the intestine (Figure 2).

Why is autophagy dispensable for Th2 cells and absolutely required for Treg cells? After all, they are all T cells and they reside in the same location. The answer might lie in the different metabolic profiles of

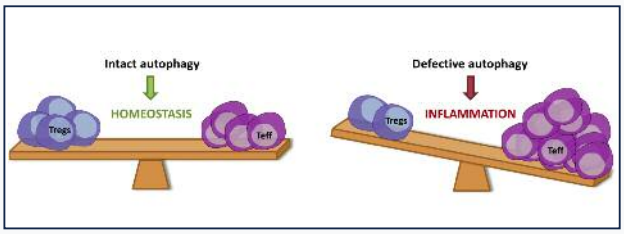


Figure 2. Autophagy maintains a healthy balance of intestinal $CD4^+$ T cell subsets. Within the intestine, the balance between pro-inflammatory effector T cells (Teff) and suppressive regulatory T cells (Tregs) is crucial to ensure homeostasis. Defective autophagy in T cells affects this balance by impairing regulatory T cells and selectively expanding the subset of effector T cells known as Th2 cells.

these two T cell subsets. Indeed, our data indicate that intestinal Treg cells show increased utilization of lipids for energy provision and that autophagy is necessary for this metabolic switch. In contrast, Th2 effector cells prefer to use glucose to maintain their metabolic demands and autophagy does not affect this pathway. Thus, autophagy differentially impacts the metabolic profiles of regulatory and inflammatory T cells in the gut [5].

Our work offers insight into how defects in a fundamental process such as autophagy might precipitate chronic inflammation. Eventually, it is hoped that understanding the functional link between autophagy, regulatory and effector T cells may provide a valuable insight into the underpinnings of not only IBD, but also other chronic inflammatory disorders, and will identify new frontiers for therapeutic intervention.

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Making a gift to the Dunn School

The Dunn School owes its existence to a philanthropic gift, from the Trustees of Sir William Dunn, and over the years has been the beneficiary of many acts of philanthropy, not least from those who have worked here. Any gift made to the Dunn School helps to further research here, whether it is made to support a specific initiative such as the ones described in this newsletter, or at the discretion of the Head of Department.

If you would like to make a gift to the Department this year, please use the gift form enclosed with this edition of *Fusion*. Please make sure that you have completed a gift aid form so that we can reclaim tax on your gift, and note that if you are a higher rate tax-payer, you can also set your gift against your tax liability for the year. All gifts made to the Dunn School from the USA are also fully tax-deductible, when made through the University's 'giving vehicle' there, the *Americans for Oxford, Inc* organization.

Photograph courtesy of Judie Waldmann

The Macrophage: A Cell Type for All Seasons

David R. Greaves

Of all the cell types of the innate immune system, it can be argued that the macrophage is the most versatile and most important. Neutrophils kill pyogenic bacteria, eosinophils repel helminth worms, mast cells degranulate (more often than not inappropriately) and dendritic cells present antigens to lymphocytes. While macrophages play a central role in defense against pathogens they do so much more besides. Our appreciation of the role of macrophages in developmental biology, inflammation and tissue homeostasis owes much to monoclonal antibodies developed in Oxford, lineage tracing techniques and a range of transgenic reporter mice.

A brief history of the macrophage

Many 19th century pathologists had seen the tell-tale signs of recruitment and activation of macrophages within foci of chronic inflammation. These included the lipid-engorged foam cells in diseased arteries that gave rise to Rudolph Virchow's lipid imbibition hypothesis of atherosclerosis and the multinucleate giant cells of Theodor Langhans that are the classic histological hallmark of *Mycobacteria* infection.

However, it was the comparative zoology studies of Elie Metchnikoff (1845–1916) that gave us a better appreciation of how multicellular organisms respond to tissue injury and the potential for infection. During a family vacation in Sicily, Metchnikoff made his now famous series of observations of a rose thorn inserted into a transparent starfish embryo using his light microscope. Metchnikoff made two key observations, (1) the attraction of motile cells towards the focus of tissue injury (a process we now call chemotaxis) and (2) the engulfment (or phagocytosis) of bacteria and debris by specialized cells that Metchnikoff first referred to as macrophages. In the late 1930s Ebert and Florey developed an intravital microscopy system in the Dunn School and used this system to make insightful observations of monocyte recruitment and macrophage (histiocyte) phagocytosis in mammalian systems.

While biochemists toiled away in cold rooms purifying humoral factors such as complement components, antibodies and cytokines, significant progress in cellular immunology had to await the development of monoclonal antibodies that specifically labelled monocytes and macrophages in bone marrow, blood, lymph and tissues. The Dunn School was quick to adopt the hybridoma fusion techniques of Köhler and Milstein and apply them to key questions in immunology. As the MRC Cellular Immunology Unit identified leukocyte antigens, a new Dunn School recruit from the Rockefeller University in New York began to isolate a range of monoclonal antibodies that specifically identified macrophages in normal tissues.

Siamon Gordon and his team developed monoclonal antibodies that identified the scavenger receptor (SR-A), important for macrophage uptake of modified low density lipoprotein (LDL), the CR3 complement receptor, and sialoadhesin *inter alia*. Perhaps the most famous Dunn School anti-macrophage antibody was one cloned by Jonathan Austyn while he was a DPhil student in Siamon's laboratory. The F4/80 monoclonal antibody was shown by Andy McKnight to recognize a 7 transmembrane spanning protein with an extended N-terminal domain that is found on nearly all tissue-resident macrophage populations.

Expression of the F4/80 antigen together with a handful of other antigens such as CD68 (recognised by monoclonals developed in Oxford by David Mason), allowed us to appreciate the distribution of the many and varied cells of the mononuclear phagocyte family, such as Kupffer cells in the liver and microglia in the parenchyma of the brain.

The many faces of macrophage activation

In the 1960s, classic papers by George Mackaness, who had read for his DPhil in the Dunn School under Florey, showed that resistance to intracellular bacteria such as *Mycobacterium tuberculosis* and *Listeria monocytogenes* could not be transferred by serum. Rather host defense required cellular immunity. Mackaness showed that pathogen-specific T cells recruited and activated macrophages in a process called delayed type hypersensitivity. Mackaness' work preceded purification of protein signaling molecules called cytokines that drive cell mediated immunity, notably interferon gamma (IFN-γ) and interleukin-12 (IL-12). In 1992, Siamon Gordon's team reported that macrophages treated with another cytokine, Interleukin-4 (IL-4), adopted an alternative form of macrophage activation, different from that seen following activation with bacterial endotoxin (LPS) or the cytokine IFN-γ. More than 20 years later, macrophage biologists are still debating the importance of macrophage polarization between so-called M1 anti-bacterial macrophages induced by LPS and IFN-γ and M2 wound-repair macrophages, induced by IL-4.

Macrophage biology in the Greaves laboratory

I joined the Dunn School with a strong interest in chromatin structure, the regulation of cell type specific gene expression and generating transgenic mice. In Siamon Gordon's lab, I identified DNA sequences in and around the human CD68 gene that directed macrophage-specific gene expression. Many groups have since used our hCD68 gene expression

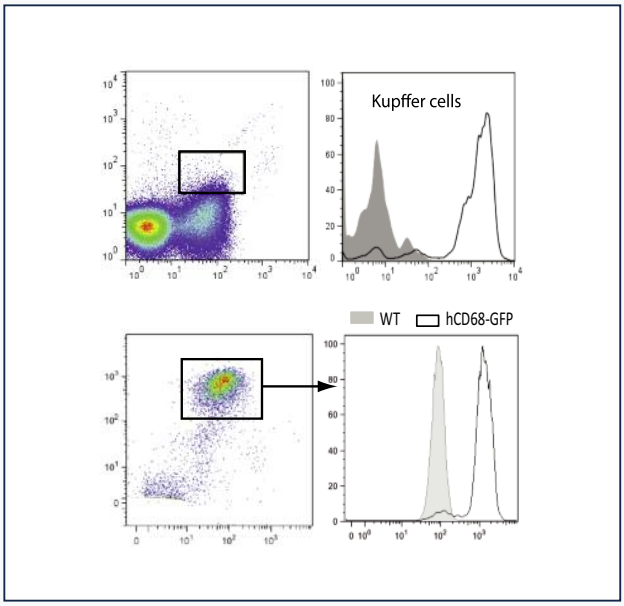

Figure 1. *Tissue macrophages of hCD68-GFP transgenic mice. Single cell suspensions of liver and bronchoalveolar lavage of wild-type (WT) and hCD68-GFP transgenic mouse lung were analysed by flow cytometry. Live cells were gated for CD45⁺ cells and analysed for macrophage marker expression (F4/80, Siglec F and CD11b – left panels), then analysed for Green Fluorescent Protein (GFP) expression (right panels). Data courtesy of Asif Iqbal, Eileen McNeill and David Greaves.*

cassette to drive high-level expression of a wide range of transgenes in macrophages *in vivo*. Detailed characterization of our hCD68-GFP transgenic mouse line showed green macrophage populations in embryonic, foetal and adult tissues. Figure 1 shows an example of GFP^+ Kuppfer cells and alveolar macrophages analysed by flow cytometry. An important feature of our hCD68-GFP transgenic mice is that their GFP^+ monocytes retain high-level GFP expression during their differentiation into macrophages. This unique feature has allowed us to explore monocyte recruitment and macrophage differentiation during the development of atherosclerotic lesions in the $ApoE^{-/-}$ mouse model of atherosclerosis.

Returning to the original observations of Metchnikoff, my lab has developed real time methodologies to quantify both macrophage chemotaxis and phagocytosis. Figure 2 shows an example of bone marrow-derived macrophages engulfing unopsonised *E. coli* bacteria. The superior quantification of macrophage phagocytosis in our new assay comes from repeated imaging of the same group of cells, sophisticated image analysis and pH sensitive dye labeling that means phagocytosed particles only manifest when they are within the acidic pH of the phagolysome. We are now using this system to study macrophage phagocytosis of apoptotic cells — a process called efferocytosis — a key initiator of inflammation resolution and tissue repair.

The resolution of inflammation

Tissue resident macrophages play a key role in the initiation and amplification stages of inflammation by sensing non-self molecular patterns and synthesizing protein and lipid inflammatory mediators. Current work in my laboratory concentrates on identifying endogenous signaling pathways that modulate the host inflammatory response. We have recently shown that the cannabinoid receptor CB2 plays a previously unappreciated role in regulating the mobilization and recruitment of neutrophils in response to an inflammatory stimulus in the skin. Using chemical biology approaches we hope to exploit our original observation to develop new anti-inflammatory drugs and hopefully enhance tissue repair processes. This will be very challenging but there can be no more inspiring place to do this work than the Dunn School of Pathology.

Classic Papers and Further Reading

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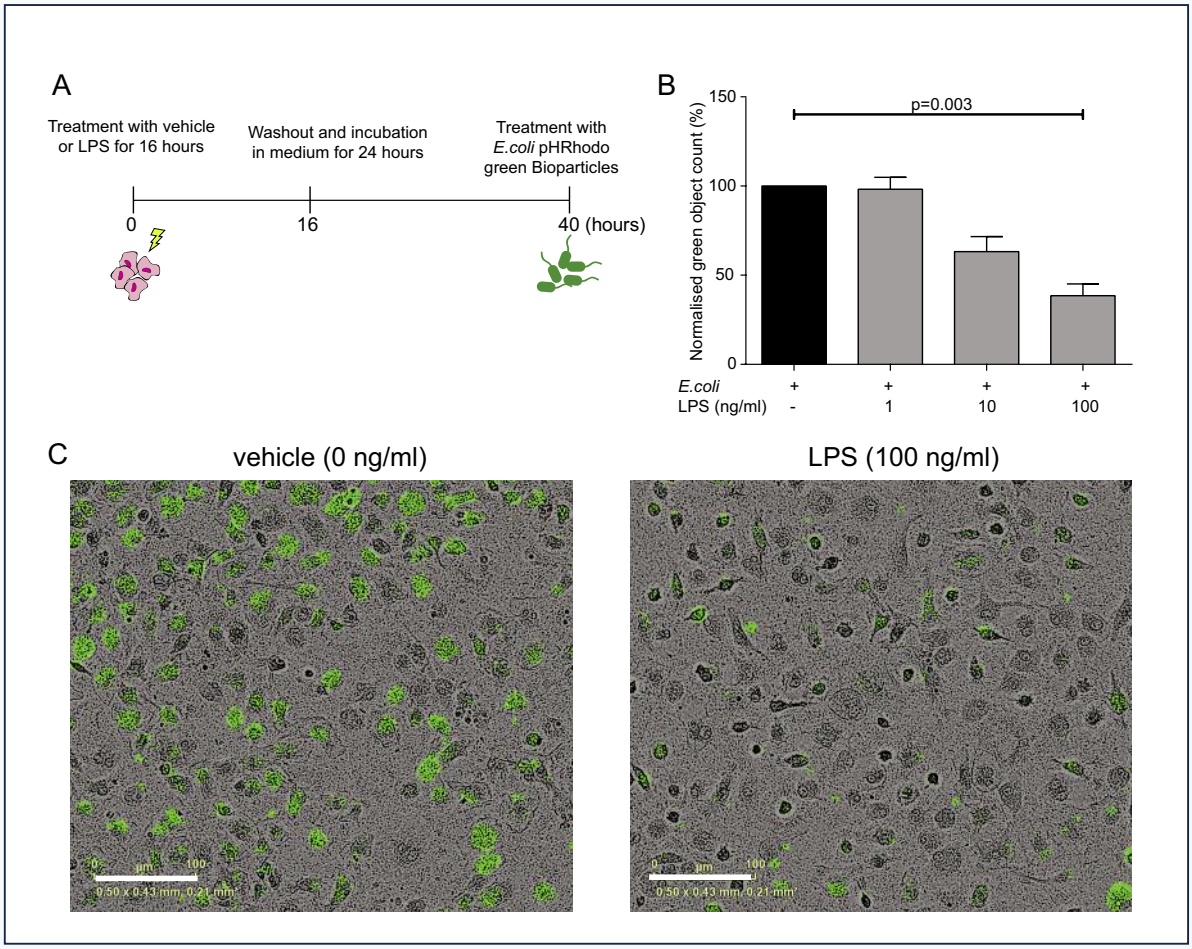

Figure 2. *Macrophage phagocytosis of unopsonised* E coli *bioparticles. Murine bone marrow derived macrophages (BMDM) were incubated with and without bacterial endotoxin (LPS) for 16 hours, then washed and incubated in culture medium for 24 hours before incubation with a phagocytic meal of pHRhodo labeled killed* E. coli *(Panel A). The phRhodo dye only gives green fluorescence at an acidic pH, which is found in the mature phagolysosome within the macrophage. Imaging of green fluorescence every 10 minutes for 60 minutes allows real time measurement of macrophage phagocytosis (data are shown as mean from 3 independent experiments). Data courtesy of Theo Kapellos, Asif Iqbal and David Greaves.*

Fuelling Immune Tolerance

Duncan Howie

Both fuel and function

In the late 1920s Otto Warburg, a German physiologist and future Nobel laureate, observed that cancer cells depend on glycolysis, a relatively inefficient form of metabolism, to generate energy rather than the more efficient oxidative phosphorylation [1]. Glycolysis generates ATP, the fuel 'currency' of the cell at a rate of 2 ATP per glucose molecule as opposed to around 36 per glucose via mitochondrial oxidative phosphorylation. It is likely that cancer cells use this mode of metabolism because by-products of glycolysis are used by the cells as building blocks for new DNA and cell membranes for dividing cells. This seminal observation led to the current idea that, in addition to generating energy, different pathways of metabolism are also used by cells for other purposes. Ninety years later, we now have a growing understanding that cellular metabolism is flexible, and that many pathways of metabolism have multiple roles in altering cell function, with metabolic enzymes fulfilling alternative non-metabolism related roles, a phenomenon known as 'moonlighting'. Lymphocytes have different metabolic modes depending on the environment in which they find themselves and their specific role. Altering metabolism in lymphocytes and myeloid cells can have profound effects on their activation and functional polarisation. We have recently become interested in T lymphocyte metabolism as it relates to immune tolerance.

Immune tolerance requires regulatory T cells expressing Foxp3

The therapeutic immunology group, headed by Herman Waldmann, investigates the process of immune tolerance and how it functions with the goal of harnessing these mechanisms for new clinical therapies. Over the last thirty years, using mouse models of skin transplant rejection, the group has shown that a subset of white blood cells termed regulatory T cells (Treg) are essential for induction of therapeutic tolerance to foreign skin grafts [2, 3]. These cells normally act like the policemen of the immune system, inhibiting other lymphocytes from attacking 'self' whilst allowing these cells to eradicate pathogens. Treg express a transcription factor, a DNA binding protein, called Foxp3 which controls the expression of over 1000 genes. This protein is sufficient to 'program' cells to become regulatory. The importance of Foxp3 is well illustrated in a thankfully small number of boys in whom the gene for Foxp3 is mutated (Foxp3 is located on the X chromosome). This results in a particularly nasty and frequently fatal autoimmune condition called IPEX [4]. One of our aims is to discover what Foxp3 does to cells to enable them to become regulatory.

Foxp3 alters cellular metabolism

Like most branches of cell biology, we rely heavily on advanced instrumentation and reagents to answer our questions. We have used a variety of molecular approaches to investigate how Foxp3 reprograms T cells. One major advance in our methodology was the development of transgenic mice into which a reporter 'tag', a human cell surface molecule called CD2, was genetically inserted into the Foxp3 gene. In these mice, Treg are tagged on their surface with the human protein and can be easily identified and purified for study. We also use retroviruses to shuttle the Foxp3 gene and its mutated forms into T cells to study its effects, and mice in which the Foxp3 gene has been deleted. Using these tools we have studied gene expression and the proteome controlled by Foxp3 with microarray technology and the Dunn School's state-of-the-art mass spectroscopy facility. These approaches led to a surprising discovery; that Foxp3 enhances expression of multiple protein subunits of the electron transport system, the mitochondrial complex responsible for oxidative phosphorylation. Using a Seahorse™ bioanalyzer, an instrument that measures extracellular flux of protons and oxygen, we found that Foxp3 enables cells to increase their rate of oxidative phosphorylation, although Treg perform glycolysis at a rate similar to other T cells. Intriguingly, Foxp3 enhances the cell's flexibility to use multiple fuel substrates leading them to take up fatty acids from their surroundings at a much higher rate. We can measure the uptake of glucose and fatty acids into different lymphocyte subtypes and analyse the role of Foxp3 in this process using an Imagestream flow cytometer. This instrument enables us to simultaneously look at 10 different fluorescently labelled cell parameters in addition to taking a high resolution photomicrograph of each cell being analysed (Figure 1).

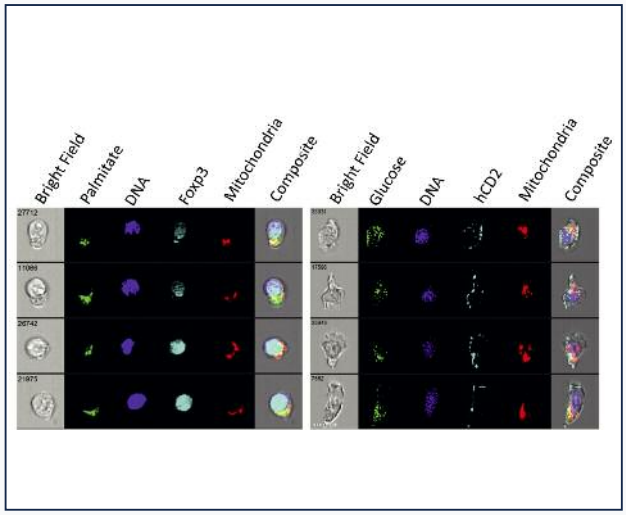

Figure 1. *Imagestream™ analysis of murine Treg fed BODIPY-labelled palmitate or 2-NBDG, a fluorescent glucose analogue. Cells were co-stained with 7-AAD to stain DNA, anti-Foxp3, mitotracker deep red to stain mitochondria and anti-human CD2 to stain the cell surface reporter tag for Foxp3 expression.*

Fatty acids are a very rich fuel: palmitate produces 106 molecules of ATP compared to 36 via glucose metabolism through oxidative phosphorylation in the mitochondria. Treg use these fatty acids, the basic subunits of fats, to power their enhanced oxidative phosphorylation.

Metabolic 'moonlighting' in T cells

Treg, in addition to having altered metabolism compared to conventional T cells, can also reprogram the metabolism of dendritic cells, innate cells responsible for presenting antigen to T cells and inducing their activation. Steve Cobbold observed that Treg induce in dendritic cells upregulation of catabolic enzymes which metabolise essential amino acids. The resulting microenvironmental deficiency of essential amino acids is sensed by bystander conventional T cells by an intracellular sensor called mTOR. This results in inhibition of mTOR in these bystander cells and transcription of the Foxp3 gene. By altering the metabolism of dendritic cells, Treg can therefore induce local deficiencies in nutrients that leads to induction of fresh Treg, a process we termed 'infectious tolerance' [5].

Flexibility in fuel choice by Treg may also give them a survival advantage in microenvironments deficient in glucose or amino acids. Fatty acids, particularly long chain fatty acids, those with chains of 16-18 carbons, are toxic at high physiological concentrations to many cells including T cells. We found that a surprising consequence of increased fatty acid metabolism in Treg is that they are relatively protected from fatty acid-induced cell death compared to conventional T cells, possibly contributing to their longevity.

Conventional T cells also have a few metabolic moonlighting tricks up their sleeves, with metabolic enzymes and their products influencing mRNA translation, transcription factor phosphorylation and epigenetic modification. For example, many of the enzymes of the glycolysis pathway have been shown to have RNA binding activity. GAPDH binds to interferon gamma (IFN-γ) mRNA and inhibits its translation. T cells engaging in anabolic metabolism, where GAPDH is fully active in the glycolysis pathway, release the inhibition on IFN-γ allowing it to be translated. In resting cells, the rate of glycolysis slows and the 'extra' GAPDH available is able to inhibit IFN-γ [6] (Figure 2). Pyruvate kinase, the final enzyme of glycolysis, has been shown to phosphorylate STAT3, a transcription factor which transduces signals downstream of pro-inflammatory cytokines in T cells. Epigenetic control of T cell genes may also be controlled by products of the citric acid cycle, such as the co-enzyme NAD+. So it is likely that the type and intensity of metabolic activity of T cells may alter their responses to cytokines and their functional phenotype in a multitude of ways.

Final thoughts

Recent advances in technology and instrumentation available to immunologists to study immune cellular metabolism and nutrient sensing have triggered a resurgence of interest in the role of metabolism in all aspects of immunity. The challenges ahead will be to decipher how cells integrate all their environmental cues to control their metabolism through nutrient sensors like mTOR and transcription factors such as Foxp3. Knowledge of these mechanisms may form the basis of future therapeutics to manipulate immune tolerance. Pharmaceutical manipulation of metabolism in T cells to increase or diminish Treg numbers and functions may well provide a means to enhance tolerance and boost anti-cancer immunity respectively.

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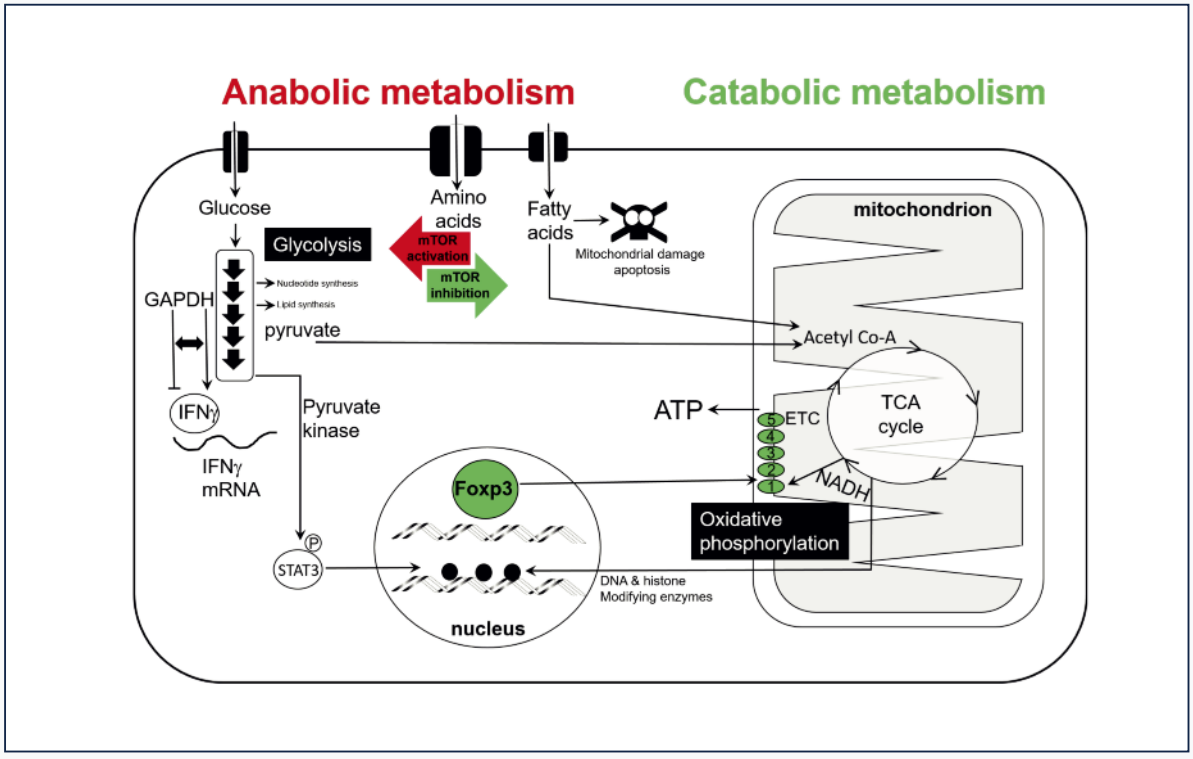


Figure 2. *Foxp3 expression and mTOR inhibition, via low intracellular nutrient levels, drive cells towards catabolic metabolism. Foxp3 drives up-regulation of the electron transport chain and oxidative phosphorylation driven by fatty acids. mTOR activation favours anabolic metabolism. Enzymes of the glycolysis pathway have 'moonlighting' functions in controlling STAT3 phosphorylation and IFN-γ translation. ETC= electron transport chain.*

As one of Herman Waldmann's first PhD students, Steve Cobbold's career has focussed on better understanding transplant rejection, as part of which he was actively involved in the development of CAMPATH, a monoclonal antibody recently licenced for the treatment of multiple sclerosis (see News Items). Having recently suffered from kidney failure, Steve was given a kidney transplant and found himself on the receiving end of the very drug he had helped develop. Here, he tells of his personal journey back to full health.

A Taste of One's Own Medicine: The Ultimate in Clinical Translation?

Steve Cobbold

It was Christmas 1979, and together with my PhD supervisor Herman Waldmann we started the work that led, over many years in both Cambridge, Oxford and big Pharma, to the approval of CAMPATH (or alemtuzumab). This is now used all over the world to reduce organ transplant rejection and to treat Multiple Sclerosis. At this time, a young surgeon (Peter Friend) worked with us towards his MD, and Peppy Rebello became his research assistant (and later, my wife).

The first signs of kidney problems

It started with blood in my urine after a bout of *Campylobacter* from a dodgy Chicken Madras. After urological investigations it was declared that I had a harmless "foot stomp haematuria" caused by playing too much squash. Unconvinced, I spoke to some rheumatologist friends who suggested IgA nephropathy, but as there was no treatment, I ignored it. I was then 28.

Twenty years later… in Oxford

After a routine health check and a 3+ for protein in the urine, a kidney biopsy confirmed IgA nephropathy (a sort of autoimmune disease). Two years later my kidney function was down to 14% and I briefly went on the transplant waiting list. I then had Christian prayer and my function miraculously improved to >30%, and with the help of >20 different medications, I remained stable for the next 12 years.

Heading towards dialysis?

My kidney function suddenly deteriorated in November 2014 (triggered by a chest infection) and I went back on the transplant waiting list. I was also anaemic as my kidneys had stopped making EPO, so I was injecting the cyclists' drug of choice, making it (just) possible to keep playing squash. Peppy (now my wife) (Figure 1) offered to donate me a kidney but was not a match. However, we found a reciprocal matched pair in a UK-wide pooled donation scheme at the first attempt (a sort of online dating for kidneys). It was touch and go whether I needed to prepare for dialysis, but everything went smoothly and the transplant took place on 17th August 2015.

Figure 1.

The transplant: 5 days in hospital

Peter Friend (now the Director of the Oxford Transplant Centre) performed both Peppy and my surgeries. The first thing I remember as I awoke in the recovery room was a nurse saying "the CAMPATH is going in now"! After the operations both Peppy and I recovered well, with no pain (no need for the magic morphine button). I now had a 20cm half-moon wound sealed with superglue and 3 kidneys (they don't remove the old ones). The transplanted kidney started producing urine immediately but creatinine and potassium levels took longer to normalise. They got me up a day later but I was very tired. They wheel around noisy trolleys all night and surround you with machines designed only to provide a continuous beeping sound or alarms! The anaemia rapidly improved, so no more EPO. Peppy went home on day 3, but visited with my parents on day 4, when I was allowed out to the coffee shop (plus my "yellow handbag"). I was supposed to be pushed in a wheelchair, but I ended up pushing my mum instead. After being discharged I had to avoid infections and be monitored by the Churchill clinic 3 times a week.

2 weeks post-transplant

Neither of us could drive yet, so Peppy's sister came from Canada to act as chauffeur for the clinic visits — a nightmare for her with all the roundabouts and roadworks. We went for walks every day to help reduce the risk of bladder infections. I was feeling really good – neither Peppy nor I realised quite how ill I had become. Everyone kept saying how pink I looked: my skin had been covered in grey-brown blotches plus scars from all the itching (high phosphate), but all that has completely cleared up. The doctor has allowed me to start driving again (this normally takes 4–6 weeks)!

4 weeks post-transplant

My new kidney is working well at about 40% of the function of a normal person (Figure 2). Although the donor was anonymous, I think the kidney was from a female over the age of 50 (female kidneys go into vasoconstriction due to the loss of oestrogen, raising

blood pressure). The half-life of a living donor female to male kidney transplant in the absence of any other complications is apparently 27 years; so hopefully, it should keep me going for a while.

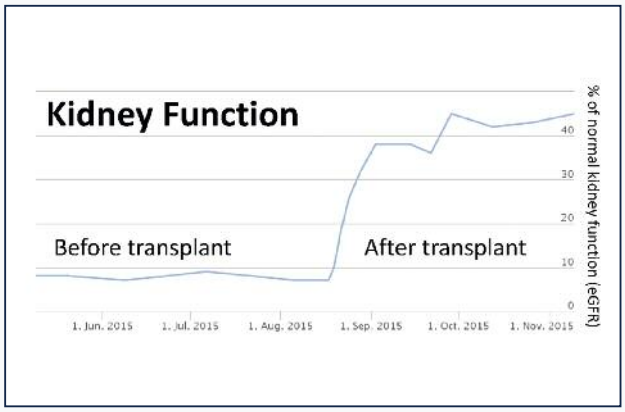


Figure 2.

The stent, which held open the new ureter where it joined the bladder, had to be removed (because of increased risk of infection). The surgeon explained this would be a minor operation using a camera inserted through "natural channels" under local anaesthetic.

6 weeks post-transplant

Peppy was signed off as healthy — but more tired than before — and I am now back at work. Life after transplant has definitely improved. Peppy notices the biggest changes — my breath and clothes stank like a gent's urinal before, but now all she can smell is my after shave! I can also sleep the whole night without hourly trips to the bathroom (kidney failure doesn't always mean you stop peeing — I couldn't retain water so I had to keep drinking to keep up). Peppy, however, is disappointed that the new kidney has failed to cure my snoring!

8 weeks post-transplant

I am now down to only 8 different medications, although I will have to be on immunosuppression indefinitely. I had my first "gentle" game of squash with the over 70's Scottish Masters champion. We played 4 games, leaving me a bit stiff the next morning, but nothing untoward. I can now use my espresso machine (10 years old but rarely used pre-transplant due to the high potassium in coffee). I can even do something useful in the evenings rather than falling asleep in front of the TV.

Two weeks before Christmas

Cytomegalovirus (CMV) has been detected in my blood. It hides away in most of us, but can reactivate when the immune system is suppressed and potentially damage various organs, including the kidney transplant itself. So I had to stop one of my immunosuppressive drugs to help me fight the virus, increasing the risk of graft rejection. I also started a strong anti-viral medicine, a side effect of which means I have no blood neutrophils to protect me from bacteria. Two days before Christmas I get called in urgently to check for rejection. Thankfully it was a false alarm and I avoided having to spend Christmas on intravenous steroids. Peppy developed a nasty cold, but I somehow managed not to catch it. We had a mostly quiet Christmas and New Year apart from further blood tests to follow up the rejection scare and to make sure the CMV remained under control.

17th February 2016

My new kidney is 6 months old. I suffer from multiple mouth ulcers, but they clear up after another adjustment of immunosuppression. I am back up to the top box in the University squash and playing for the Club team in the Oxfordshire leagues. We also have a fantastic week of spring skiing in Val D'Isere (with lots of factor 50 sun cream, to reduce the melanoma risk).

Final thoughts

So far, so good, but there are still risks of further serious infections, heart disease, diabetes and cancer. The possibilities of graft rejection or failure also remain very real. This is why we still need basic research into transplantation tolerance, when the graft is no longer seen by the immune system as "foreign", eliminating the need for continuous immunosuppression.

I have perhaps done the ultimate in clinical translation. I would like to think that CAMPATH contributed to the success, but as Geoff Hale, who coordinated the academic trials on the drug, once remarked: "so many people around the world were praying that we can never be quite sure". However, Figure 3 may speak for itself!

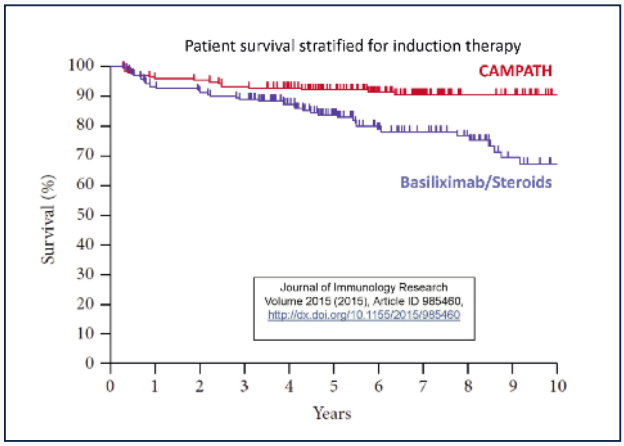


Figure 3.

Disclosure: Stephen Cobbold is the Professor of Cellular Immunology at the University of Oxford and receives royalties from Genzyme/Sanofi for the sales of CAMPATH, which is now marketed under the trade name Lemtrada, for the treatment of relapsing, remitting multiple sclerosis.

Interview with James Gowans

James (Jim) Gowans first joined the Dunn School in 1947, finally leaving to take over at the helm of the Medical Research Council in 1977. During the period he spent in the department, he was the first to demonstrate the recirculation of lymphocytes, which led to our current understanding of the dynamics of the immune response. During a recent interview with* Fusion*, he described the background to his research and some of the personalities who contributed, sometimes unwittingly, to his success.

Given that the recirculation of lymphocytes is so central to our understanding of immunology, perhaps you could briefly outline how your discoveries came about.

Well it's really very simple. When I came back from to the Dunn School, Florey asked me what I wanted to do, and I think I said 'immunology', since that was what I had worked on at the Pasteur Institute. Florey was appalled, and said 'I don't want to know what -ology you want to work on; I want to know what experiments you're going to do when you come into the lab tomorrow!' That was typical of Florey, and I was completely floored since I didn't have the slightest idea. 'Well', he said, 'there is one problem that concerns lymphocytes, that we've worked on for some years in the lab and which has become known as the 'mystery of the disappearing lymphocyte". If large numbers of small lymphocytes enter the blood each day, an equal number must leave the blood to maintain a constant level. This was at a time when there was no immunology attached to lymphocytes, but everybody was talking about their fate. The idea early on was that lymphocytes were short-lived cells and must die, and one theory proposed that they die in the gut. Then Yoffey came along claiming that they don't die but go to the bone marrow where they turn into other blood cells. Yoffey's ideas were based entirely on transitions that he alleged you could see between lymphocytes and other cell types. I remember Yoffey coming to the lab with a bunch of slides and showing them to Florey, who found it difficult to conceal his contempt for this work as it flew in the face of everything he believed about pathology (you do experiments, you don't just peer down a microscope or cut up dead bodies), Florey being a physiologist by training. So Florey said to me that I should work on the same project, since many people in the laboratory had already blunted their wits on the disappearing lymphocyte, so there was no reason why I shouldn't share the same fate…

Anyway, Florey handed me a paper I hadn't seen which was by Bollman, an experimental surgeon, in which he described a technique for cannulating the thoracic duct of a rat and Florey said 'there you go, you do that, it looks easy'. So I mastered the technique, which turned out to be not all that difficult. It was very interesting because two people, called Mann and Higgins working in the States, used Bollman's technique and found that very large numbers of lymphocytes emerge from the thoracic duct over a 24 hour period, but during successive days the numbers fall right down. They commented on this observation and said that it may be due to exhaustion or the rats could be stressed, or maybe the cells were recirculating. So the idea of recirculation wasn't new. I thought the thing to do was to infuse the cells back into the animal and see what happens. So I got a pump to slowly infuse at what I thought were physiological rates, the cells that were coming out of the thoracic duct back into the blood stream and see if that influenced the output. And it did! The trick was not to inject a great bolus of cells in one go but to slowly infuse them, otherwise they all finished up in the lungs.

How did your work progress after that original finding?

That led to the first of three papers on lymphocytes. The second one was to prove that the extra cells that corrected the decline in numbers, were the same ones as I had injected. That was quite easy to show because if you infuse tritiated thymidine at the same time as you infuse the cells, to radioactively label proliferating cells, then the lymphocytes that come out are a mixture of large cells with lots of grains over them and many small lymphocytes with no grains over them, which seemed to show that they were not new cells. That was the basis of the second paper we published in the *Journal of Physiology.* And then in the third paper, we showed that the route of recirculation was through the lymph nodes by way of specialised vessels called high endothelial venules.

So that's how it happened: it was entirely due to Florey that I did those experiments. Once the project was launched he took an interest in it, although he didn't guide me at all. I invented the apparatus and designed all the experiments. He once put his head around the door and said 'Well done!' and then disappeared again, and that was that! One didn't have deep discussions with him: he couldn't stand hot air. You didn't speculate with Florey!

So why do you think he was so successful as a scientist?

I think he was a good experimentalist. He knew how to do an experiment. Everybody believed what he said, and he had a sort of rectitude about him which one tried to emulate. The currency was 'the single telling experiment', then he'd listen. But if you just tried to get clever with him he'd say 'Go and do some experiments and then I'll listen'.

So how did your success alter the course of your career?

Well, finally I had become an immunologist, which I'd never really professed to be, since I am basically a physiologist at heart. That transition was due to a lifelong friendship with my mentor, Peter Medawar. I wanted to transfuse lymphocytes from one animal to another as it was easier than putting the animal's own cells back in. In those days we just had out-bred strains of rat, so I wrote the Peter Medawar and said 'Look, I want to transfuse these cells from one animal to another. Should I first make the recipients tolerant of the cells?'

He invited me to visit him at UCL, where he was Professor of Zoology. When I arrived, he was typing away and I'll always remember his first words: 'It's very tiring writing undying prose' he said. He was very interested in my work because he too had worked at the Dunn School and his wife Jean had also worked there and published a paper on lymphocytes. He was a great admirer of Florey, who I think was one of his PhD examiners and said that his thesis read more like philosophy than science, which, he knew, was far from a compliment! So I got to know Peter, and his answer to my query was not to induce tolerance but to make some inbred strains instead, which took quite a long time. It was under his influence that we showed that small lymphocytes were antigen-sensitive cells which were able to initiate immune responses. Later my old friend Jacques Miller identified two classes of small lymphocytes, B and T cells.

So were your findings about lymphocyte recirculation thought to be significant at the time, or was it only with retrospect that people appreciated their importance?

Oh no! I remember describing the findings to Murdoch Mitchison who said they were very interesting but not biologically illuminating, so I felt very down about it. It was probably a view shared by many, that our findings were a piece of detective work, a bit of problem solving, but didn't seem to have a great deal of relevance, until, that is, Burnett came along. Burnett's idea of clonal selection fitted in rather nicely, because it seemed that recirculation of lymphocytes provided a selective mechanism for immune responses in regionally stimulated nodes. Before that time, Burnett had actually written in one of his earlier books that the small lymphocyte doesn't seem to have any role in immunology…!

What was it like working at the Dunn School during the early days of immunology?

I had a good time in the Dunn School. I shared a room with Henry Harris, who was doing RNA work, but nobody else was working on immunology. My early papers were published under my name alone; in those days' single author papers were not unusual. However, in one paper, my DPhil student Julie Knight, was a co-author. She showed that the large, dividing lymphocytes in lymph migrated into the wall of the gut and developed into plasma cells. There was nobody in the Dunn School to talk to about immunology. I talked to members of the Medawar lab at UCL. The transplantation people had become interested in lymphocytes because it seemed that they were involved in rejection. Medawar suggested I should go to the States to do a lecture tour, which gave me good exposure there. The bit I remember best was in Harvard where I got to know Joe Murray who was a kidney surgeon and the first person to transplant kidneys between identical twins, for which he won the Nobel Prize.

What changes have you seen in science during your lifetime?

Well, science has completely changed now and molecular biology and genetics dominate the whole of biology, which they certainly didn't in my day.

When I started working on lymphocytes there were probably only a dozen people in the world working on them and most of those were in the States or Scandinavia. So there were periods when I knew everything they were doing because we used to swap information by letter. It's very difficult to choose a field now that's populated by so few people. If you go into a field where lots of people are working, even if you work under somebody good, it's very hard to break into the big time: even if you are invited on your own to meetings as a self-propelled group leader, you can stay a post-doc for a long time because tenured posts are so rare. So I think that career prospects in scientific research aren't as good these days.

It was a great luxury for me to spend my life with no teaching and no administration, just research. I felt very privileged, especially since I actually got paid for doing it! But although at times I thought I might be kicked out, I never felt insecure because there were so many other opportunities: I could go back to being a doctor, I could teach and so on. These days I think people feel insecure, and social habits have changed. The financial demands come much earlier and the pay for post-docs is not sufficient to meet them. Science is much more competitive now and the need for recognition can dominate lives in a way that it didn't in my day. Naturally, I wanted to be recognised but it didn't seem to be quite so difficult in those far-off days!

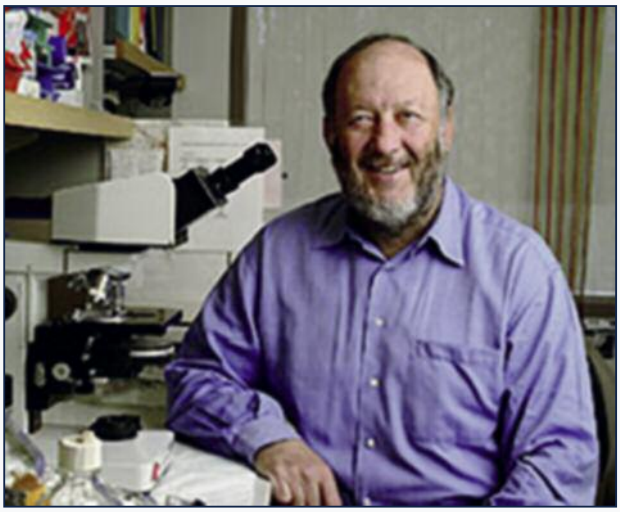
Irving Weissman is Professor of Pathology and Developmental Biology at Stanford University and is Co-Director of the Stanford Institute of Stem Cell Biology and Regenerative Medicine. During the early stages of his career, he spent some time in Jim Gowan's lab at the Dunn School where he contributed to our current understanding of the role of the thymus in the biology of lymphocytes. Here he reflects on his time in Oxford and his recollections of Jim as a mentor.

Jim Gowans: Recollections of a Mentor and Friend

Irving Weissman

When I entered the laboratory at the Dunn School in 1964 I had not yet finished Medical School, and went there to test whether the thymus made new immunocompetent cells (as opposed to making hormones — after all, the thymus was a gland!). In the lab at that time were Sam Strober, a Harvard Medical Student, Bill Ford, a DPhil student, and a few others. Jim still did the rat thoracic duct cannulations and I was devising how to label interphase and dividing thymic lymphocytes to test if they made it out of the thymus and emigrated to the lymph nodes, spleen, Peyer's patches, and thoracic duct lymph.

Although Jim was very much held in awe by us, he didn't act like I thought very proper Oxford Dons would act. One Sunday, I came into the lab and Bill Ford was devastated. He had been cannulating rat spleens to measure the circulation of small lymphocytes into and out of the spleen, and to test whether the immune response came from lymphocytes already resident in the spleen, or the blood stream. After many hours setting up the preparation he made a mistake and ruined the prep. He had slammed his hand down on the counter, and the mettler balance on the counter fell and broke. By the time I had gotten in, Bill was figuring out how he could return to a career in Medicine, as he had blown his research career. I told him it should be fine, but I hadn't been in the lab long enough to know how Jim would react. I couldn't convince Bill that he still had a career. But when I got in the next day, Bill was beaming. Jim had told him that of course his career wasn't over. And Bill showed, much to my surprise, that it was the lymphocytes in the blood, not the spleen, that initiated an immune response to blood-borne sheep red blood cells. This made Jim's discoveries of recirculation of clonally pre-committed antigen-specific lymphocytes even more relevant, as it was the circulating cells — I would guess T cells now — rather than tissue resident lymphocytes that were important to initiate adaptive immunity. (Upon antigenic stimulation the responding cells lose their recirculation homing receptors for the blood vessels in the lymphoid organs or tissues, and as we and others showed, express receptors for blood vessels in incipient inflamed tissues.) There are probably few immunology students in the current generation who know these critical experiments and why they are important; and how or why long-term memory lymphocytes lose the inflammatory homing receptors to be replaced again by those used by recirculating naive lymphocytes. But in these days of immunotherapy with activated T cells, and the hopeful transfer of memory T cells for lifetime immunity to cancers or viruses, it is good that Jim was not an imperious professor, but our mentor, and still to this day, my friend.



I finished my experiments, met Jim in New York City on the way back, where he had just done a mini-sabbatical with Jonathan Uhr during which he showed that recirculating lymphocytes could carry immunological memory, and reported on my many results. When I finally wrote them up, I sent the draft bearing his name to Jim but he generously wrote back saying that I had done the experiments, and I should, therefore, report them. After leaving Jim's lab, Bill went on to many great studies of his own, but died tragically while on sabbatical in Australia some years later.

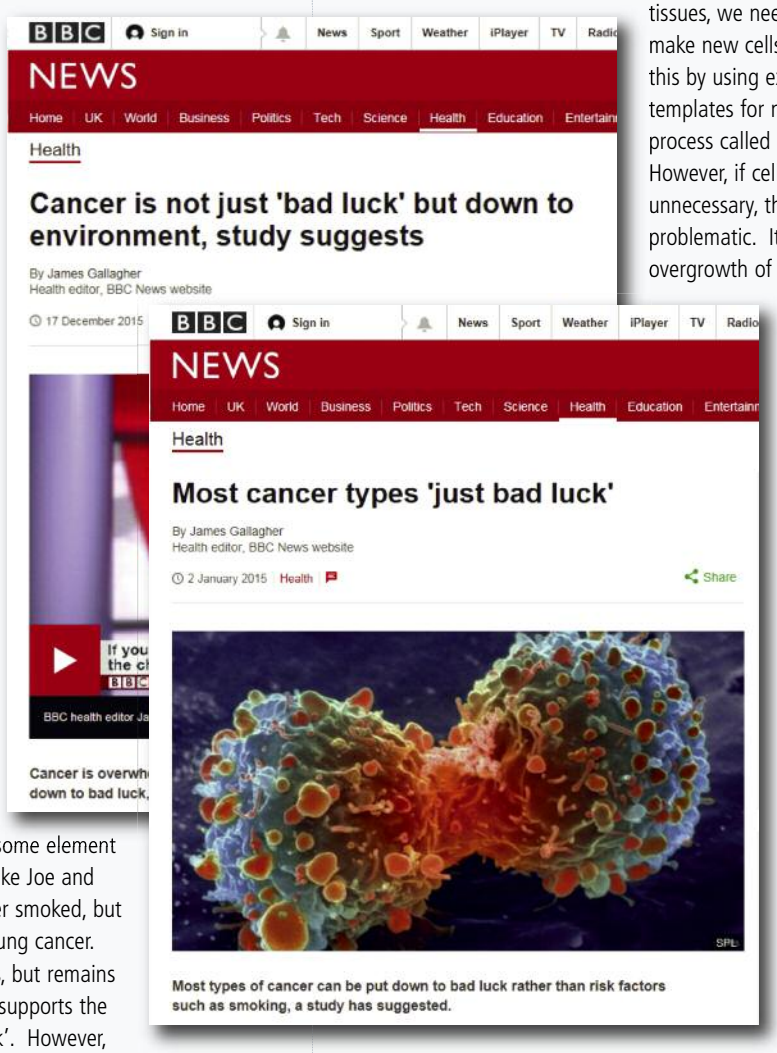
Jessica Hardy, a DPhil student in Chris Norbury's laboratory, was recently awarded a prize by the Biochemical Society in its 2016 Science Communication competition. Here we reproduce, with permission of* the Biochemist *magazine*, her winning entry which examines how the public might make sense of the conflicting messages it receives about the causes and risks of cancer.

Cancer: A Disease of Bad Luck, or Bad Lifestyle?

Jessica Hardy

Cancer. It's an emotive word and a dreaded diagnosis. We all know someone affected by this horrible disease and, quite understandably, we all want to know: what causes cancer, and is there anything we can do to stop ourselves from getting it?

This is one of the most burning public health questions of modern times, but it's pretty difficult to find a clear answer. Take these two headlines, both published on the BBC News website in 2015 [1, 2] and both based on scientific studies: "*Most cancer types 'just bad luck'*" and *"Cancer is not just 'bad luck' but down to environment, study suggests"*.



So, which one is it? Can we throw caution to the wind, keep the 40-a-day smoking habit and indulge in a daily fry-up, knowing that our risk of getting cancer is beyond our control? Or can we completely eliminate our cancer risk by filling our lives with superfoods and daily workouts?

As you've probably guessed, the answer lies somewhere between these two extremes. There is clearly some element of 'bad luck' in developing cancer. Take Joe and Mike, who are both 61. Joe has never smoked, but sadly he's just been diagnosed with lung cancer. Mike has smoked heavily for 45 years, but remains healthy. This might seem unfair, and supports the idea that Joe's cancer is 'just bad luck'. However, it's well established that smokers are much more likely to develop lung cancer than non-smokers, and it would be unwise to completely dismiss the influence of lifestyle on cancer risk.

But what proportion of cancer risk could be considered out of our control, and how much influence does our lifestyle really have? To tackle this question, it's important to understand how cancer develops. Cancer is, in short, a disease caused by excessive division of cells. Cells are the functional building blocks of our tissues and organs, and in order to grow, and to repair or replenish parts of these tissues, we need to be able to make new cells. Our bodies do this by using existing cells as templates for new ones, in a process called cell division. However, if cells divide when it's unnecessary, this can be problematic. It can lead to an overgrowth of cells, forming a tumour — a mass of rogue cells which don't work properly, and which disrupt the function of the affected organ. Left unchecked, these deviant cells ultimately evolve the ability to spread within the body and seed new tumours, eventually damaging vital organs and causing death.

The question is, then, what makes cells start to misbehave and divide when they shouldn't? The key lies in our DNA — the genetic code found in every cell, which provides the instructions that make the cell work correctly. This includes, for example, the code to make molecules that regulate cell division. The problems begin when this code is altered in some way — a process called mutation. This can be thought of as miscopying or changing the code, much as someone might make a mistake when typing up a handwritten document. Let's imagine a

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secretary, Bruce, typing up some meeting notes. He's usually very accurate, but occasionally a mistake creeps in. This might be harmless, and might not change the meaning of the sentence. For example, he might type 'We must not DISPOZE of hazardous waste in the yellow bin' instead of 'We must not DISPOSE of hazardous waste in the yellow bin'. OK, he misspelt a word, but it doesn't really matter. Sometimes, though, the mistake might have dangerous consequences. He might type 'We must NOW dispose of hazardous waste in the yellow bin', rather than 'NOT'. That will cause trouble! The same can be said for copying the DNA code into a new cell during cell division. When representing the DNA code, we use four letters — A, T, C and G — to signify the four building blocks of DNA. A letter 'T' in the code could, for example, be miscopied as a 'C'. Depending on which part of the code is affected, this may have little effect on the instructions, or it might change the behaviour of the new cell.

These DNA mutations happen very occasionally, by chance, every time a cell divides. This represents the 'bad luck' aspect of cancer. If enough chance mutations accumulate in important places in the DNA, enough instructions might be changed to make a cell divide continually or develop characteristics that support tumour growth — the so-called 'hallmarks of cancer' [3]. However, there are many factors which can increase the chance of these mutations arising. Let's consider Bruce's typing again. If he types his notes after having a few pints of beer, or after getting only two hours of sleep, he's much more likely to make mistakes. Analogously, smoking, the most notorious risk factor for cancer, greatly increases the chance of DNA mutations, as the chemicals in cigarettes can directly react with DNA, leading to changes in the code. Equally, too much sun exposure greatly increases skin cancer risk because UV light induces chemical reactions within DNA that can alter the code.

You may be wondering why then, if we understand how mutations can arise and lead to cancer, there are still such conflicting reports on how much of cancer risk is down to 'bad luck'.

The article entitled *'Most cancer types 'just bad luck'* [1] was based on a study that addressed the question of why some organs, such as the bowel, are more prone to cancer than others, such as the brain [4]. The researchers found that this was partly explained by the number of dividing stem cells in each organ. The bowel is constantly shedding and replenishing its lining, with the new lining being supplied by a continually dividing population of cells known as stem cells. The frequency of cell division in the bowel is, therefore, much higher than in some other organs, such as the brain, where there isn't such a large and continually dividing stem cell population. More cell division and copying of the DNA means more chance for mutations to be introduced by miscopying. The authors used mathematical models to show that around two thirds of the variation in cancer rates between organs is explained by differences in stem cell division rates, and therefore suggested that 'random' mistakes in DNA copying during stem cell division are the underlying cause of the majority of cancers [4].

Unfortunately, the media headline that 'most cancers are bad luck' led many to announce with delight that they could keep their unhealthy habits and stop worrying. Whilst this bold headline may have had some element of evidence backing it, being based on the two thirds figure from the study, it overlooks the quite significant one third which are seemingly influenced by external factors. It also ignores the important suggestion that environmental factors might contribute to these seemingly 'random' mutations that accumulate during cell division.

In fact, another study, which analysed some of the same stem cell division data [5], led to the second headline — *'Study suggests cancer is not 'just bad luck'* [2]. This study argued that just because a tissue with more cell division is more prone to cancer-causing mutations, it doesn't mean that these are 'random' mistakes. Environmental factors could easily contribute to mistakes made during cell division, just as they can cause mutations in non-dividing cells. The researchers used different mathematical models based on this idea, and also looked at the types of mutations found in different cancers to try and figure out what proportion look like those often caused by external factors. Their analysis, contrary to the first study, suggested that only 10–30% of cancers are due to 'random mistakes', with the majority involving some lifestyle influence [5].

You might ask how two rigorous scientific analyses based on the same data can give such different conclusions. The reality is, the maths is complex — the groups constructed different mathematical models based on slightly different assumptions and predictions in order to analyse the available data. The real answer may be somewhere between these two figures, and as we research more into the factors that promote DNA mutation and cancer growth, these models and estimates will continue to improve. But one thing is for sure: there is certainly some, probably fairly significant, contribution of environmental factors to our risk of developing cancer, and some proportion of risk that is innate to our biology.

The take-home message is that nobody is immune to cancer. DNA mutations will happen — it is a fact of life. And sometimes, although thankfully rarely, a particularly unfortunate cocktail of mutations may arise that leads to cancer developing. There is nothing we can do that will guarantee this won't happen. However, we can certainly stack the odds in our favour, reducing the frequency of these mutations and the chance that cancer will develop. Research is continually improving our understanding of which lifestyle factors contribute to cancer development, and although we are still bombarded with confusing and sometimes conflicting reports on what we should and shouldn't do, there are some very well-supported recommendations as detailed by Cancer Research UK [6]: don't smoke, drink less alcohol, eat lots of fruit and vegetables, maintain a healthy weight and avoid excessive sun exposure. It might sound boring, but these really are some of the best things you can do to try and keep your DNA, and your body, as healthy as possible.

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HISTORY CORNER

A Lifetime of Penicillin

Eric Sidebottom

February 12th 2016 was the 75th anniversary of the first injection of penicillin into Albert Alexander, the moribund policeman with overwhelming infections of *Staphylococcus* and *Streptococcus* who had been chosen as appropriate for the first clinical trial of the new drug (Figure 1).

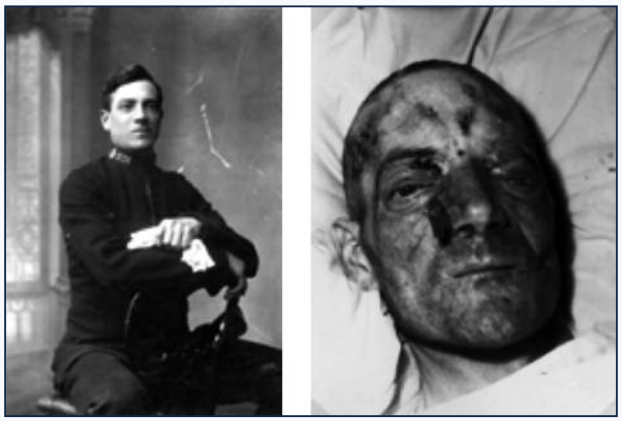

Figure 1. Albert Alexander before his illness (left), and shortly before treatment with penicillin (right).

Since only about 5% of the world's population is over 75 very few of us have lived without antibiotics. I have claimed that the introduction of penicillin was the most important medical advance of the 20th century and arguments still rage on how significant the introduction of the 'antibiotic era' has been in the dramatic rise in world populations that have occurred in the last 75 years (from about 2.5 billion in 1950 to 7.3 billion now).

The wonderful story of the discovery and development of penicillin as the world's first antibiotic has been told many times, (most recently in a small book by David Cranston and this author, currently in press, which concentrates on the role of the 'unsung hero', Norman Heatley, in the work). The world knows that Alexander Fleming in 1928–29 described the inhibition of the growth of staphylococci — and so 'discovered penicillin' — and that in 1945 he shared the Nobel prize with Howard Florey and Ernst Chain for 'the discovery of penicillin and its curative effect in various infectious diseases' but the world in general does not understand that Fleming had no part in developing penicillin for clinical use. That work was done entirely in Oxford by Howard Florey's team in the Sir William Dunn School of Pathology.

It was late in 1938 (about the time of my birth!) that Florey and Chain decided to begin a research program into anti-bacterial substances. Both had experience of the anti-bacterial enzyme lysozyme and they decided to widen that experience. Chain surveyed the literature and rediscovered Fleming's 1929 paper in the *Journal of Experimental Pathology* (a journal of which, coincidentally, Florey was an Editor) which described his experiments with the *Penicillium* fungus and its ability to inhibit the growth of some important pathogenic bacteria such as *Staphylococcus* and *Streptococcus.* Chain persuaded Florey that they should start their new program by repeating and extending Fleming's work. They recruited various members of the current laboratory staff to help them. Crucially this included Norman Heatley, a biochemist from Cambridge who had been working with Chain for about three years.

The team quickly confirmed Fleming's findings and encountered the difficulties of extraction and purification of the active component, penicillin that had plagued his attempts to progress the work. However, since, unlike Fleming, the team had a wide breadth of scientific expertise they succeeded in isolating and purifying the active component of the 'mould juice'. This enabled them to test the power of penicillin to treat infection in living animals. The first such experiment was conducted on Saturday May 25th 1940 with eight mice. That experiment, and the many that soon followed it, proved beyond reasonable doubt that penicillin could cure infections in animals caused by several much-feared bacteria such as *Streptococcus, Staphylococcus,* and *Clostridium.* The results of those experiments were published in the *Lancet* on August 24th 1940.

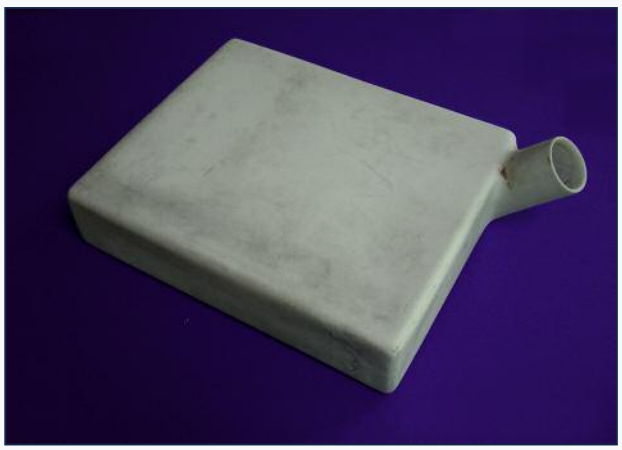

Figure 2. Example of a ceramic bedpan for the culture of *Penicillium*

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THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY
is a department of the University of Oxford
website:
www.path.ox.ac.uk

CONTACTS:
Matthew Freeman
Head of Department
Sir William Dunn School of Pathology, South Parks Road Oxford OX1 3RE
matthew.freeman@path.ox.ac.uk

EDITORS
Paul Fairchild
Tel: (44) (0)1865 285751
email: paul.fairchild@path.ox.ac.uk

Eric Sidebottom
Tel: (44) (0)1865 285751
email: eric.sidebottom@path.ox.ac.uk

Florey realised that the next step would be to test penicillin in human patients but since, as he stated, "humans are 3000 times larger than mice" and no pharmaceutical company could be persuaded to undertake the manufacture, the Dunn School would effectively have to be turned into a factory. That this became possible was due largely to the ingenuity of Norman Heatley who decided that a custom-designed ceramic bed pan would be the best culture vessel for such manufacture (Figure 2). The Potteries firm of James Macintyre agreed to make the 'bedpans' and the first batch of 186 was collected by Heatley on December 23rd 1940. They were cleaned and sterilised on December 24th and seeded with *Penicillium* spores on Christmas morning. I am not aware that anyone, writing about the development of penicillin, has used the phrase 'a wonderful Christmas present to the world' but that is what this first large scale culture became. It also became apparent that a team of technicians would be needed to 'farm' the *Penicillium* cultures and so a team of up to 6 girls was recruited for this purpose (Figure 3).

Florey decided that by early February enough penicillin would have been prepared to do the first human clinical trial and so the Professor of Medicine at the Radcliffe Infirmary, Leslie Witts, was consulted. He recommended that Charles Fletcher, a young research physician, should be responsible for selecting suitable patients and administering the penicillin. Hence on February 12th 1941 Albert Alexander became the first human to receive an injection of an antibiotic to treat his overwhelming septicaemia: the so-called 'antibiotic era' was launched.

The high point of the antibiotic era was probably reached in the mid 1960's when the now famous/infamous statement of William Stewart, the United States Surgeon-General was published. He is reputed to have claimed, in a report to the US Congress, that "It is time to close the book on infectious diseases, and declare the war against pestilence won". But there is no primary source for this statement and more recent authors have claimed that he neither believed this nor stated it in those words. Nevertheless there was, at that time, a huge optimism that our ability to identify, isolate and even synthesize new antibiotics would slowly eliminate major infectious diseases.

It is interesting to note that Fleming, in his Nobel lecture in 1945, warned of the dangers of not using the new antibiotics responsibly and it is now clear that his worst fears have materialized. We are now living in, what I hope, is the worst phase of the "pessimistic era" of antibiotics. This was ushered in by the publication of Robert Bud's book *"Penicillin, triumph and tragedy"* in 2009 in which he reported how inappropriate use of antibiotics had allowed microbes to quickly develop resistance to almost all the currently used antibiotics and he warned that unless we "changed our ways" the situation would get progressively worse. The current government's Chief Medical Officer Dame Sally Davies has also repeatedly emphasized the dangers we now face and urged that much more research is needed to identify new anti-bacterial and anti-viral agents. Being an optimist I think that the worst fears of the disasters ahead will not be realized and I am confident that today's scientists will find new ways of preventing and treating the new infectious diseases that are almost certain to appear, building on the seminal success of the Oxford team at the Dunn School of Pathology.

Figure 3. Members of the Oxford team.

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