fusion

THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

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Focus on DNA

When Science Met Strictly

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SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

Welcome

It's impossible not to focus my comments this year on the coronavirus pandemic and how it has affected the Dunn School over the past 18 months. It has been a bleak time for us all, but we developed the concept of Covid 'silver linings' – if one looks hard, there have been positives and, as we seem to be emerging from the worst, it is good for us to reflect on them.

As soon as the potential scale of the pandemic become clear, we established a crisis management group – the Dunn School Silver Group. Our first decision was to define an overall strategy that would steer our responses: our twin principles were to focus on the safety of everyone in the department, while also minimising the damage to our research. An early tactical decision was also to maintain frequent and open communication with everyone. Although we have inevitably had to develop policy on the fly – there's no playbook for this – the strategy has stood up well to the changing situation and has been effective in guiding our operational decisions.

One of the very clear silver linings has been experiencing the famous spirit of the Dunn School under such stressful conditions. Our hallmark collegiality, mutual support, and tolerance has helped control the many inevitable wellbeing issues that arise from the pandemic. These qualities have also been of real practical value. The goodwill of everyone has underpinned our response to the challenges of making the department as Covid-secure as possible, allowing us to become one of the first University departments that permitted the return to laboratory work. There are far too many people to thank personally here, but I would like to pay tribute to the collective efforts of the Graduate Student Association, the Postdoc Association and the Lab Managers Group, as well as our fantastic facility and maintenance staff.

Of course, even having successfully reopened the Dunn School in early June last year, many of us have continued to spend much of our time working from home. Like the rest of the world, we have become all too familiar with virtual meetings – a potential silver lining when thinking about future work patterns and the environment, but also with the negative consequences associated with too little real human interaction. Science, like all of us personally, does not thrive without the myriad informal meetings, conversations and social opportunities of normal life. Indeed, one of our biggest challenges has been how to retain the social aspects of work, at a time when there's much less chance to meet than usual. We have also been conscious that a significant number of our colleagues have had additional challenges such as home-schooling, looking after and shielding elderly or vulnerable members of the family, or just loneliness.

I am proud that the Dunn School has contributed to Oxford's globally prominent scientific response to the pandemic. In an initiative spear-headed by William James and Becky Moore, and described later in this edition of *Fusion*, we established Oxford's first Category 3 laboratory licensed to grow live SARS-CoV2 virus. This gave us an important role in the early stages of Oxford's vaccine development effort. Several other labs urgently refocused their research to addressing fundamental questions about the mechanism of viral infection, the cellular response and possible novel therapeutics. More broadly – another silver lining – I think that the pandemic has highlighted to everyone, well beyond our usual audiences, the importance of studying the fundamental mechanisms that underlie human disease. This bodes well for the research future of the Dunn School.

Indeed, 'all this' has not stopped us keeping our eyes on the future. Despite the additional complexities associated with lack of travel, and the need for distancing, we are undertaking an ambitious programme of recruitment this year. We have three Statutory Professorships and at least two Associate Professorships to fill, as well as aiming to bring in at least one new externally funded early career fellow to start their first group. I'm also very pleased to welcome Sumana Sanyal, who joined us from the University of Hong Kong as a new Associate Professor and who is featured in a Spotlight article later in this edition. Her arrival in January 2020 was especially timely, as she researches RNA viruses and the cellular response to infection. Within weeks of arriving, she had incorporated SARS-CoV2 into her programme.

I am also very pleased to announce that, in a collaboration with Brasenose College, and the support of very generous donors, we have endowed an Associate Professorship in cell and molecular biology. This will ensure an enduring link with Brasenose, and a permanent research and teaching post in the department. Indeed, as we begin to plan for the Dunn School centenary in 2027, I have the hope that we will be able to endow more of our academic posts: this kind of philanthropic support has perpetual impact on the future success of the Dunn School.

Finally, although 2027 sounds far away, we have started to think about how to mark the centenary. What a hundred years it's been; and what a future we have ahead of us! It's too early to know exactly how we will celebrate but, if endowing posts and studentships is the goal that would have most strategic impact, what would give me the greatest pleasure would be to build connections with the whole extended Dunn School family, past and present. Later in this issue we include a note on how you can subscribe to our new alumni mailing list, which will complement the annual edition of *Fusion*. Our efforts to streamline our communications in the build up to our centenary, and make them fully GDPR-compliant, extends to *Fusion* too. If you would like to continue to receive *Fusion* in the future, the law now requires that we receive your active consent, which you can provide simply by emailing us on alumni@path.ox.ac.uk or by posting the enclosed postcard to us. This is, of course, only the beginning of what we have planned for the next few years, so please do stay in touch and watch this space!

Matthew Freeman

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Honours for Current Dunn School PIs

Despite the constraints of lockdown and the impact wreaked on research by the pandemic, PIs at the Dunn School have continued to be recognised for their ground-breaking work with the award of various honours.

In April 2021, **Elizabeth Robertson** was elected to the US National Academy of Sciences, one of only a small number of scientists from outside the United States to have received such recognition. Her work, featured in the last edition of *Fusion*, has spanned several decades and spawned many new insights into aspects of developmental biology.

Tanmay Bharat, an early career scientist, has likewise been recognised for his pioneering work on antimicrobial resistance, achieved through studying the structure and function of extracellular surface layers that surround and protect bacteria. He was awarded the 2021 Eppendorf Award for Young European Investigators, established to acknowledge outstanding contributions to biomedical research in Europe. Furthermore, his work was recognised independently by the Biochemical Society with the award of the 2022 Colworth Medal.

However, in something of a coup for the Dunn School, the Biochemical Society also honoured **Ivan Ahel** with the 2022 GlaxoSmithKline award which recognises eminent bioscientists and exceptional early career researchers. Ivan's ground-breaking work on ADP ribosylation has yielded important insights into basic cell and molecular biology and holds promise for novel approaches to medical intervention. Recipients of the Biochemical Society awards have been nominated by their peers and are endorsed by a panel of respected scientists, drawn from a variety of disciplines, attesting to the excellence of the research performed by either laboratory.

Recognition for Former Members of the Dunn School

Two former members of the Dunn School have been honoured for their seminal contributions to medical science.

Malik Peiris, Professor of Virology at the University of Hong Kong and Director of the Centre for Immunology and Infection, obtained his DPhil at the Dunn School under the supervision of James Porterfield. His work has recently been recognised by the John Dirks Canada Gairdner Global Health Award, conferred upon him for his outstanding contributions to understanding the origins and options for control of emerging infectious diseases. His work on avian influenza has led to an effective monitoring and surveillance programme for avian and swine flu but he is best known for his contribution to identifying the coronavirus behind the outbreak of severe acute respiratory syndrome (SARS) in 2003, which has acquired even greater significance in the light of the ongoing SARS-CoV2 pandemic.

Wilf Jefferies studied for a DPhil in molecular immunology at the Dunn School and is currently a Principal Investigator of the Michael Smith Laboratories at the University of British Columbia as well as being Head of Immune Oncology at the Vancouver Prostate Centre at Vancouver General Hospital. He has been recognised for his seminal discoveries on mechanisms underpinning cancer immune surveillance and immune-editing by T lymphocytes, as well as the role played by dendritic cell cross-priming in triggering anti-tumour

immune responses. His innovative approaches to cancer immunotherapy have led to his election as a Fellow of the National Academy of Inventors (NAI), the highest professional distinction afforded to eminent academic inventors. Professor Jefferies will be joining a prestigious group of only 13 academic inventors in Canada to be elected as Fellows of the NAI and the first Canadian immunologist to be inducted.

Maria Leptin Appointed Head of the European Research Council

After a rather turbulent period in its history, the European Research Council (ERC) has elected Maria Leptin to take the helm as its President, with effect from October 2021. Maria has had a close association with the Dunn School, having assumed the role of Visiting Professor in Cell and Developmental Biology in 2018. As part of her role, Maria has held workshops for aspiring group leaders in the department providing helpful insights into how to prepare compelling grant and fellowship applications, an essential milestone in any scientific career. And few know better how to successfully navigate the complexities of an academic career path: having served as Professor in the Institute of Genetics at the University of Cologne since 1994 and as Director of the European Molecular Biology Organisation (EMBO) from 2010, Maria has led successful laboratories both in Heidelberg and Cologne and is well known for her work on topics as diverse as developmental biology, wound healing and innate immunity to infection. As President-elect of the ERC, she will wind down her research activities to focus wholly on her new role which she envisages will involve her petitioning European leaders to increase the available budgets for the forthcoming Horizon Europe program and increasing the emphasis on outreach to convey the importance of basic, knowledge-based research.

Farewell to a Trusted Neighbour

For over 50 years, the Tinbergen Building on the south side of South Parks Road has provided a stark contrast to the manicured lawns and red brick façade of the Dunn School's old building, standing opposite. Renowned for its brutalist architecture and designed by Sir Leslie Martin, the Tinbergen Building housed the departments of Zoology and Experimental Psychology for over 50 years and was the University's largest teaching space, with the only lecture theatre capable of seating 800 people. The discovery in 2016 that the building was riddled with asbestos led to its precipitous closure and the displacement of up to 2000 academic staff. The decision not to attempt a refurbishment of the existing building but to build a new institute in its place, secured the Tinbergen Building's fate. During the course of lockdown, the existing structure was, therefore, demolished (Figure 1) and the asbestos carefully removed, ending the Dunn School's long-term association with a trusted neighbour. The new Life and Mind Building that will replace it (Figure 2) is reportedly the largest building project ever undertaken by the University and will provide space for the Department of Experimental Psychology while combining Zoology and Plant Sciences into a single new Department of Biology. The recent announcement that the Ineos-Oxford Institute for antimicrobial resistance will likewise be based in the Life and Mind Building provides one of many opportunities for members of the Dunn School to collaborate with our new neighbours. The new building is due to open in September 2024.

Figure 1. Demolition of the Tinbergen Building during lockdown 2020 (Pictures courtesy of Tim Davies).

Figure 2. Artist's impression of the new Life and Mind Building, due to open in September 2024.

Not Strictly Science

In October 2020 Martin Parnov Reichhardt, published his latest paper on the protein SALSA, an abundant component of the human mucosal innate immune system. This publication comes with a curious timing, as Martin is currently occupied as a professional dancer on the Danish version of the TV-show *Strictly Come Dancing*.

Originally from Denmark, Martin started his PhD studies at the University of Helsinki, Finland in 2010. He arrived as a Postdoc at the Dunn School in the lab of Professor Susan Lea in January 2016. Just prior to arriving in Oxford, Martin retired from a more than 20-year long career as an international competitive ballroom dancer. During his sports career, Martin ranked in the top 20 on the World Ranking, and with 7 National Championship Titles as a professional dancer, Martin built a strong reputation as a dancer before retiring. Though fuelled by his interest in science, Martin is one of the few postdocs that has left Oxford, not for another academic position, but for a job as a dancer on *Strictly Come Dancing*. Though dancing and science seem worlds apart, Martin emphasizes the similarities. Creative thinking, relentless dedication and no small measure of optimism is required in both fields. He always felt dancing provided a re-charge of the brain for science, while the academic work provided a well-earned rest for tired feet.

During his time in Oxford, Martin worked with X-ray crystallography and cryo-EM, and published structures of a novel complement inhibitor, as well as the SALSA protein. After Oxford, Martin was initially heading for a position at the Karolinska Institute in Sweden. However, Denmark called him home, and Martin first appeared in the famous TV show in September 2019. Martin is still involved with scientific work through the University of Helsinki, and recently published a paper showing altered SALSA levels in the intestine of prematurely born infants. However, after arriving back in Denmark, Martin has switched his main career to management consulting. He currently holds a position with Boston Consulting Group, which luckily allows him a leave of absence once a year to follow his passion and dance on *Strictly*.

Photograph Tim Davies

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 yearshas 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.

The Silence of the Labs: Photodiary of a Pandemic

Wayne Swan

Dunn School Facilities Manager, Wayne Swan, was keen to capture the eerie emptiness of the Dunn School during the Covid-19 Spring of 2020. Although the department never fully closed, with Covid-19 research underway and a small amount of other research activity continuing, the department was largely empty with labs deserted and the previously manicured Dunn School garden gone to seed. As the department slowly emerged from lockdown in June 2020, corridors were marked as one-way, maximum room occupancy signs were adhered to doors and café furniture socially distanced. Wayne's camera captured it all.

Rising to the Challenge: Developing Collaborations in Coronavirus Research

William James

At the beginning of 2020, it became clear that everything was about to change. As a virologist, I must confess to having had mixed feelings. On the one hand, I was filled with curiosity about the biology of this new virus that was causing such mayhem in Wuhan and intrigued to discover more about it. On the other, I was nervously reading all the reports for early signs of whether this virus had the potential to spark a pandemic that would disrupt all our lives. By the end of February, good quality reports, including that of a WHO visiting group to Wuhan, were making it clear that this virus was very dangerous indeed. Its combination of high intrinsic reproduction rate, or R0, and its ability to cause serious disease in a significant fraction of those it infected, which was fatal in about 1% of cases, placed it firmly at the disruptive end of the spectrum. At that point, work in our lab was moving along very nicely on a couple of fronts: investigations into the molecular control of neuroinflammatory responses in diseases such as Alzheimer's and Parkinson's disease, led by Sally Cowley; and work on HIV and Zika virus infection of macrophages, particularly the regulation of latency and the impact of antiviral resistance genes on macrophage metabolism. Two things seemed at that point to be increasingly likely. First, that the new pandemic would disrupt our ability to continue these lines of research in the planned way. And second, that, as virologists, we were duty bound to consider ways in which we could contribute to amelioration of some of the worst consequences of the pandemic.

Since 1985, we had run a containment laboratory at the Dunn School to enable us to work on "live" HIV-1. In 1995 we opened a new and well equipped containment laboratory suite to support the ongoing programmes of research we were doing in Oxford on HIV, including work in which I was collaborating with Siamon Gordon, Alan Williams, and Nick Proudfoot at the Dunn School. In recent years, the facility was also supporting the research of Quentin Sattentau in the Dunn School and Alfredo Castello based in the Biochemistry Department, but we judged there was sufficient spare capacity to consider devoting the majority of its space to work on the new coronavirus, SARS-CoV2. Accordingly, over Easter 2020 with huge support from Dr Becky Moore in the Sattentau lab, and Tracey Mustoe in the Safety Office, we managed to undertake the physical recommissioning required, and the development or entirely new codes of practise that resulted in clearance by the Health and Safety Executive on the 7th April. Becky and I lost no time in developing a set of standard operating procedures for the COVID-19 suite in order to support four main workstreams designed to satisfy demand for live virus work amongst our colleagues in Oxford and further afield. These included: methods to grow, quantify and quality-control isolates of SARS-CoV2 from reference sources and clinical material; neutralisation assays to determine the potency of antiviral antibodies and similar macromolecules; IC_{50} assays to determine the antiviral potency of small molecule drugs; and individualised, or 'bespoke' investigational studies.

We trained a total of 10 further colleagues* in these methods to provide the capacity to support the increasing demand for such work. By this time, all non-Covid-19-related research had shut down in the University and so we were fortunate enough to be able to identify research students, postdocs and career scientists with suitable experience who were keen to be re-trained in this way. It has been very gratifying to see the extent to which this team of people have dedicated themselves to the new work. After about two months, it was no longer necessary for Becky and me to continue to be the main technicians for this work as the team were not only fully trained but now very self-organised. As we approach Autumn 2021, the little team of Covid-19 specialists have increasingly returned to their non-Covid research, making it timely to look back on some of their achievements. In one piece of work[1], we reported a collaboration with clinical

Photographs Wayne Swan

colleagues from Oxford in which we showed that blood samples containing detectable viral RNA were nonetheless safe to use in standard diagnostic labs, as they contained no culturable virus, substantially accelerating the throughput at the hospital. In a set of papers in a related field, we have demonstrated the neutralising potency of structurally-defined monoclonal antibodies, nanobodies and chimeric proteins to the Spike protein of SARS-CoV2 that are entering further development with a view to use in patient treatment[2]. We were also able to provide confirmation that a subset of the sera from participants in the Oxford Covid-19 vaccine trial did indeed contain high titres of neutralising antibody[3]. Although the flow of routine requests for work in the facility has now diminished, we are still very excited by the work we are doing with collaborators on the neutralising nanobodies and optima's that have been recently generated and expect work in this area to continue to produce interesting and potentially useful results over the next year. More broadly, we have begun to work in detail on the questions relating to the pathogenesis of Covid-19 and particularly the role of inflammatory and immune responses in cellular damage. One important aspect of this work concerns the possibility that antibodies specific for the virus could, under the wrong circumstances, enhance the infection of alveolar macrophages and thereby result in worse outcomes. The process of 'antibody-dependent enhancement' was elucidated by James Porterfield in relation to flaviviruses at the Dunn School in the early 1980s with particular contributions from his students Malik Pieris and Jane Cardosa. We now know from small animal models, that sub-neutralising levels of anti-viral antibody enhance disease in individuals challenged with SARS-CoV2. To provide a pathophysiologically-authentic yet experimentally-tractable model for this, Sally Cowley is leading a small team who have developed an "alveolus in a dish" comprising human pluripotent stem cell-derived pneumocytes and macrophages. Using this system, we will be able to test whether particular antibodies at specific concentrations enhance infection and whether the result of virus uptake through this route generates signals of inflammation that may themselves be damaging. Clearly, as well as being of basic scientific interest, these results may be important for our interpretation of natural and induced immune responses to the virus.

*Michael Knight (Fodor group), Javier Gilbert-Jaramillo and Adam Harding (James group), Maeva Dupont (Sattentau group), Lise Chauveau (Rehwinkel group, RDM), Michelle Hill and Juliane Brun (Zitzmann group, Biochem), Marko Noerenberg (Castello group, Biochem), Peter Wing and Alan Zhuang (McKeating group, NDM)

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Silver Linings of a Pandemic

Catarina Vicente, Science Strategy and Projects Manager at the Dunn School, reflects on the first few months of her new role and how the department joined forces to manage the impact of the coronavirus pandemic

The first week of March 2020 marked an exciting milestone in my professional career. Seven years after finishing my DPhil at the Dunn School, I was due to return to the department as its first Science Strategy and Projects Manager. I knew it would be a challenge to return in a different capacity, to meet the new groups and people who had started since I left, and to discover what a Science Strategy and Projects Manager does (it was a new role that I could partially shape). It was indeed a challenge, but not in the way I had predicted. Within two weeks of my start date, I was working from home, meeting all my new colleagues virtually, and working almost full time on crisis management and planning!

To Matthew Freeman's credit, a coronavirus meeting was already on my agenda from day one. It was clear that something big was coming, and that the department needed to plan for it. Matthew gathered a small group of people that brought different points of view: Philip Cobden from health and safety, Wayne Swan from buildings, Andrew Souter from human resources, Chris Tang from a research and infectious disease perspective, and myself from science strategy and communications. In our first meeting (the only one in person so far!) we set up the structure that would manage the department's response to the pandemic in the following months.

We followed a standard crisis management structure, setting up a smaller Bronze group that met daily to manage operational issues, and the Silver group that included all of us, to meet weekly and make strategic decisions. From the outset, feedback and input would be welcome from anyone in the department.

Our discussions quickly escalated from whether it was appropriate to start restricting larger gatherings, to closing our doors to all except the few involved in COVID research. The day when we made this decision was indeed a sad one, and not what I expected to be involved in during my first month in a new job! However, this was balanced by the incredible spirit of community and support that was quickly evident. Research groups found new ways to interact and support each other online, and new virtual social groups and activities were established (such as the virtual dining classes or our NHS rainbow collage project, pictured). It was exciting to see that the old Dunn School spirit that I remembered from my student days was still very much alive! These positive initiatives were happening in contexts of personal difficulties for many who had to manage positive cases in their families, caring responsibilities alongside work and generally dealing with the feeling of physical isolation that affected us all as the country (and much of the world) went into a strict lockdown.

As soon as our doors closed, the Silver group started working on a plan to reopen. Our discussion points were varied, from almost philosophical discussions on what socially-distanced research might look like, to considering whether we could source enough hand sanitiser or surgical masks. We developed an overriding strategy early on and have stuck to it ever since: prioritising safety, minimising damage to research and valuing communication. We also devised a staged return process and started working towards it. Our efforts were not wasted, and by the time the government and the university started issuing guidance, most of our thinking was already done. We quickly jumped over the bureaucratic hurdles, to be one of the first departments in the university to reopen at the beginning of June 2020.

Our staged reopening was perhaps frustrating for some, but it was important to stress-test our measures and ensure that our building and services could cope with an increasing number of individuals who had to operate in a socially distanced way. We first only allowed those with the most pressing experimental needs to return. This was followed by two people per group, leading on to the current situation, where each group has a set maximum occupancy at any time.

Our community rose to this new challenge in an impressive way. Research groups led by their lab managers and PIs had to find new ways of working in the 'new normal', when only a limited number of people were allowed in the department at any time. Changes varied from devising shift systems to changing the layout of rooms and moving equipment. Services and workshop worked tremendously hard to change their procedures, create routes around the building and mark every room with yellow and black tape to ensure social distancing. Our facility managers had lengthy discussions with the university's health and safety team so that they could facilitate access to their essential instruments and train new and existing users in a socially-distanced way. The feedback from everyone in the department, either via our survey or direct emails, was essential. Reopening was a delicate balance between ensuring the department resumed as much experimental work as possible and ensuring that everyone felt and was safe. We probably didn't get it right every time, but we tried to adapt our departmental measures in response to feedback and, in some cases, lobbied with the university to change their guidelines.

As I revisit this piece that I started last year, over a year has passed since that first reopening. Unfortunately, our initial optimism that life and research would return to normal in the new academic year was unfounded, and with it came new challenges. Anton van der Merwe, Lucinda Risius and our Graduate Student Association had to work hard to ensure that the new students joining us in the Autumn of 2020 still felt welcome while maintaining social distancing rules. Departmental and progress seminars have continued online, and while some food provision is available, our cafeteria remains closed. Most of our administration staff continue to work primarily from home. The sustained impact of the pandemic on our research and personal lives led the department to implement several support schemes, thanks to a generous grant from the EPA Research Fund.

Yet, there were some positives. Our COVID case management protocols proved to be robust, and despite the worsening of the pandemic over the winter, we did not have to reduce our experimental work significantly, and indeed have slightly increased it. Our student and postdoc associations kept us going with an impressive array of online social events, and our virtual Christmas party included most of our well cherished traditions, including the cooking competition and a visit from Santa!

It is now August 2021. Restrictions are still in place, and unlikely to be eased overnight. Crisis management is a work in progress and we have to balance risk/benefit when cases in the community are high and not all in the department have had their two jabs. But the Dunn School is definitely a 'glass half full' department, so we are looking optimistically to the next few weeks and months and know we can count on everyone's support to make the most of any situation.
To this day it is unclear why we are called the Silver group (or maybe there is a secret Gold group that I don't know about!). However, silver is not such a bad name, as working with this group, as well as with everyone else in the Dunn School, has been a real silver lining of this pandemic. I am sure we will continue our efforts in the coming months, and I look forward to a time when the Silver group, and indeed all in the department, will be able to meet in person once again.

'The Spirit of the Dunn School' brought together photographs of the NHS rainbows produced by Dunn School staff and students and their families during the Spring 2020 lockdown. This is one example of the various initiatives that aimed at maintaining a spirit of community during this pandemic.

Elie Metchnikoff and his Legacy at the Dunn School

Mariya Lobanovska and Siamon Gordon

On 16th May 2020 we celebrated the 175th birthday of Elie Metchnikoff, the father of macrophage immunobiology. Mariya Lobanovska, a recent Ukrainian graduate in the Tang microbiology laboratory and Siamon Gordon, a Macrophage devotee at the Dunn School since 1976, wished to commemorate the occasion by drawing attention to the remarkable and prescient discoveries during his lifetime, and continuing impact on research at the Dunn School up to the present.

A brief biography

Metchnikoff was born in 1845 in Kharkiv, Ukraine. From an early age he was passionate about biology and zoology and while still a student at the Kharkiv gymnasium, he attended undergraduate lectures in anatomy and physiology at Kharkiv National University. He graduated from gymnasium with distinction and was admitted to the University to study natural sciences. His first exposure to an international research environment took place in Germany where he worked briefly before moving to Italy in 1865. His work there on developmental biology using invertebrates, attracted attention from prominent academics across Europe and shortly after his return to the Ukraine, he became a lecturer at Odessa University, a leading biology centre at the time. There he established the first Vaccine Institute in the country, which focused on bacteriology and infectious diseases. Soon after, with his wife Olga, he left for Italy, settling in Messina, Sicily. After seminal studies on phagocytosis and immunity, he received an invitation from Louis Pasteur to take up a research position at his new Research Institute in Paris. Pasteur supported Metchnikoff's theory of phagocytosis, initially rejected by many notable scientists in the field, but which became widely accepted after many years. He remained at the Pasteur Institute for the rest of his career, was awarded the Nobel Prize for Physiology or Medicine in 1908 together with Paul Ehrlich, and died in 1916.

A pioneer of science and medicine, he continues to influence many generations of investigators. A museum in his honour is situated in Kharkiv in the house that once belonged to Metchnikoff and his family. In 2020, the museum launched a website (http://mechnikov.dvorichna-vo.gov.ua/) to preserve his legacy, to educate and inspire young scientists, draw attention to the Ukrainian scientific heritage, and to promote international cooperation.

Since Metchnikoff's ground-breaking discoveries, the fields of immunology, microbiology and cell biology have made unprecedented advances over the past century. We note some of Metchnikoff's insights which continue to inform contemporary research at the Dunn School.

The Dunn School Legacy

The Past

It is fair to say that there has never been a time when research at the Dunn School did not include some influence of Metchnikoff's discoveries. This includes work by all Heads of Department, perhaps most relevant to the Florey era, which included his students Mackaness, Harris, Gowans, and their associates. Other past laboratories dedicated to the study of macrophages, to a greater or lesser extent, included Poole, Watkins, MacPherson, Gordon, Williams, Barclay and Maloy.

The Present

To illustrate the ongoing diversity of macrophage-related immunology and cell biology in the Department, we invited members of current research groups to contribute illustrations of selected research projects, collated as a 'birthday card' for Metchnikoff, were he to visit the Dunn School in 2020 (Figure 1). The range of topics, which is not exhaustive, shows that apart from their intrinsic interest in the immune and inflammatory functions of macrophages, they exploit the macrophage as a tool for studies of cell biology *in vivo*, as well as *in vitro*. Topics of current interest include phagocytosis and its variants, such as efferocytosis, HIV-1 and *Leishmania* intracellular infection, macrophage migration and secretion, and the role of microglia in age-related neurodegeneration. Macrophage and microglia differentiation after induced pluripotency is under study by the James and Cowley groups. Not illustrated, are ongoing studies on dendritic cells; these specialized antigen presenting cells (APC), closely related to macrophages and involved in lymphocyte activation and tolerance, continue to be studied by the Waldmann, van der Merwe and Fairchild groups.

Last, but not least, is the Department's current contribution to the COVID-19 pandemic. William James and Rebecca Moore in the Sattentau lab, have equipped a laboratory for University-wide studies of infection *in vitro*, including macrophages and antiviral antibody. This harks back to early studies in the Porterfield laboratory on antibody-dependent enhancement (ADE) of flavivirus infection via FcR, by Malik Peiris, a pioneer in SARS1 and COVID-19 research. The Fodor, Ahel, Sanyal and Sattentau groups pursue other studies on COVID-19 replication, potential drug inhibitors and viral entry.

The early emphasis by Metchnikoff on ageing and the microbiome in a healthy lifespan and susceptibility to pathogens has been vindicated by the COVID-19 pandemic, attesting to his remarkable anticipation of still poorly understood co-morbidities. He coined the term gerontology and introduced probiotics to promote longevity. Homer and Virgil cautioned those seeking eternal life to include inhibition of ageing in their request. The gods may not have granted Metchnikoff longevity, but he did achieve immortality.

Recommended reading

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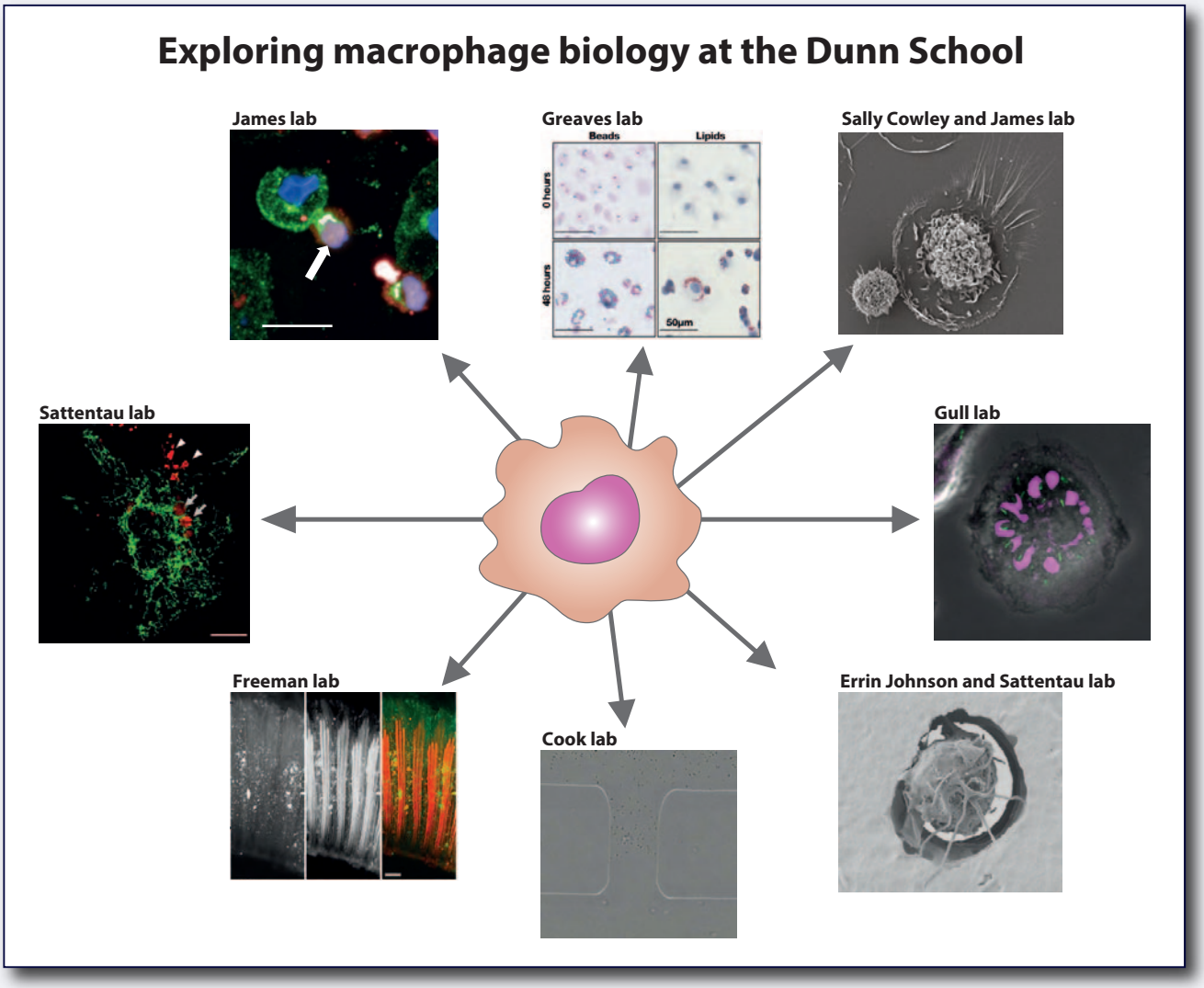


Figure 1 (clockwise): James lab: The role of microglia in Alzheimer's disease. After 3 hours of phagocytosis of pHrodo-labelled dead human neuroblastoma cell line SH-SY5Ys, immunofluorescence staining shows that TREM2 is highly recruited to the phagocytic cup (marked by white arrow) during engulfment of cells expressing the neuronal marker TUJ1 (courtesy of Hazel Roberts). **Greaves lab: Efferocytosis and substance accumulation in macrophages.** Experimental approaches and mathematical modelling are used to study apoptosis/efferocytosis in macrophages. Macrophages derived from the bone marrow of mice prior to (top), and 48 hours after (bottom), stimulation into an inflammatory state. Cells were either initially fed red and blue latex microbeads (left) or were stained red for lipid (right)[1]. **Cowley and James lab: Macrophages and stem cell technology.** SEM image of human blood monocyte-derived macrophages, which are used as a comparison for characterizing human pluripotent stem cell-derived macrophages (hPSc). Sally Cowley (Head of James Martin Stem Cell Facility) and colleagues use hPSc technology to model diseases (courtesy of Bonnie van Wildenburg and Errin Johnson, Dunn School EM Facility). **Gull lab: Parasite infection of macrophages.** Macrophage infected with *Leishmania mexicana*. The lumen of the macrophage phagocytic system (phagolysosome) is labelled in magenta. The *Leishmania* (labelled via their flagella in green) are able to infect and reside in these vacuoles causing intracellular infection (courtesy of Richard Wheeler). **Errin Johnson (Dunn School EM Facility) and Sattentau lab: Studying how HIV spreads between CD4 T cells and macrophages.** SEM image of a monocyte migrating through a porous membrane (courtesy of Errin Johnson and Cherrelle Dacon). **Cook lab: Modelling chemotaxis using macrophages.** The top chamber contains macrophages and the bottom chamber contains a chemoattractant (C5a). Both chambers are connected by a smaller channel through which a concentration gradient of C5a is established. This gradient is picked up by the cells which then start migrating through the channel (courtesy of Cyril Deroy). **Freeman lab: TNF signaling and age-related degeneration in glial cells.** The loss of metalloprotease ADAM17/TACE, that triggers the TNF inflammatory pathway, leads to abnormal accumulation of glial lipid droplets and age-related cellular damage in retinal cells. Fluorescent images of 1-day-old *Drosophila* retinas stained with BODIPY (green) and FM dye (red) to mark lipid droplets and the photoreceptor membranes respectively, showing knockdown of ADAM17 in glial cells[2]. **Sattentau lab: Recognition of 'eat-me' signals on T cells by macrophages in the context of HIV.** Image showing the phagocytosis of apoptotic T cells by a primary human macrophage. Immunofluorescence image showing T cells (red) being phagocytosed by a macrophage (green) after 3 hours contact. White arrow heads show T cell debris. Grey arrows show engulfed T cells (courtesy of Maeva Dupont).

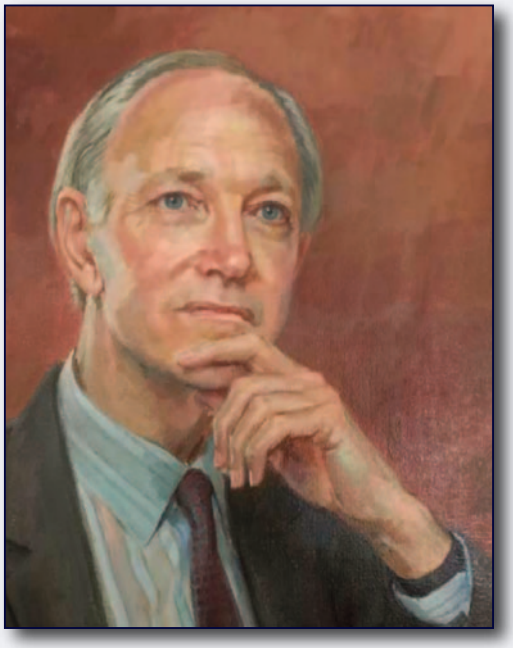
In Memoriam:
Sir James Gowans
MB BS, DPhil, KBE, FRS, FMedSci, FRCP
7th May 1924 -1st April 2020

Simon Hunt

One very hot summer day in 1947 a strikingly tall young man aged 23 mounted the fourteen stone steps of the curved staircase to the front door of the Dunn School for the first time in his life. A newly-minted graduate of King's College medical school, he had recently finished his clinical training in hospitals in south London where he lived. His purpose was an interview with Professor Sir Howard Florey about the possibility of research. Florey's secretary, Miss Poynton, ushered him up the oak staircase into that inner sanctum with the balcony overlooking the Parks which has always been the Professor of Pathology's office. Florey told him "*You won't be any good, Gowans; there's no money in it; and you're crazy…*" This remark troubled him less than it might have: he was getting used to it. He had heard exactly the same comment a month earlier by the then Secretary of the MRC, Sir Edward Mellanby, who had nevertheless brokered his introduction to Florey as '*the best experimental pathologist in the country*'[1]. Notwithstanding this laconic, antipodean style of encouragement, Florey offered a studentship to Jim Gowans. Thus began the 30 years until 1977 during which he made the Dunn School his research home, mounting those fourteen steps daily - and often nightly too[2].

Jim was the only child of John, of Scottish descent, and his wife Selma, who hailed originally from a farm in Sweden. They moved from Sheffield to Croydon in 1928 when Jim was four years old. There in south London he grew up, living through diphtheria and typhoid epidemics in the 1930s. His father was employed as a hospital diagnostic pathology technician – 'an academic manqué' with a sharp and bookish mind, as Jim once said. On his Sunday morning visits to the lab with his dad, Jim knew well the smells of tissue-block fixatives and Lysol; he fed the animals kept for immunisations; and he counted the colonies on bacterial Petri dishes. He immersed himself in Paul de Kruif's then-popular book '*Microbe Hunters*'. Later, Jim was to say "*Medicine to me had always been something laboratory-based … I quite enjoyed the clinical side of medical training. But in a positive way I always wanted to work in a lab.*"[3].

His secondary education was at the nearby Whitgift School. He was studious, and his parents were keen for him to succeed. Academically, though, he was no more than an 'average performer' (his own description). With his height advantage, he was a successful sportsman: high-jumper, hurdler and cricketer[4]. In his sixth form in 1940 the male teachers had departed to serve in the military, being replaced by Frances[5], young, female and very inspiring, who fired his enthusiasm for Biology. He gained a medical place in 1942 at King's College, making the daily commute to The Strand, participating in fire-watching duty and avoiding the buzz-bombs. He wangled his way into the famous Friday Evening Discourses at the Royal Institution, one of which in 1944 was delivered by Florey, about penicillin. They didn't meet on that occasion, but Jim was definitely impressed.



Portrait by June Mendoza, c.1990. Courtesy of the Gowans family. Copyright reserved.

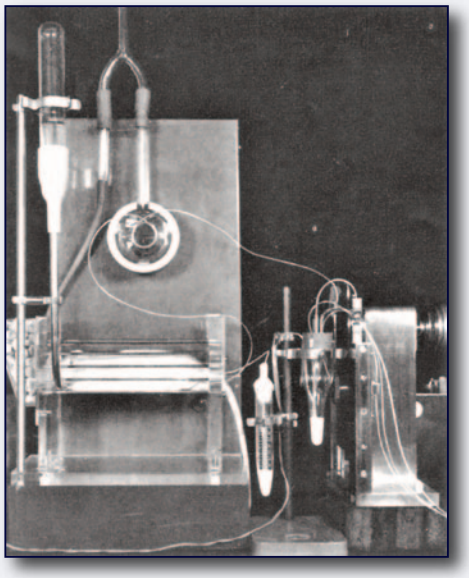
In April 1945, halfway through his medical course, he volunteered to join nearly 100 other young British medical students to assist in ameliorating the desperate situation immediately after the liberation of Bergen-Belsen concentration camp in northern Germany. He spent his 21st birthday there. They faced a horrendous scene of utter human degradation: tens of thousands of dead, dying, starving, hopeless inmates, callously treated and abandoned as disposables. In an interview recorded seventy years later[6], he vividly recalled the nauseating stench of excrement, bodily decay, and the incineration of infected materials. The hollow stare of one particular moribund prisoner in a transient, wordless eye-to-eye exchange, remained unpurgeably engraved in his memory. He and one other student were put in charge of hut number 39, housing 200 inmates. No-one knew how to effectively care for such extremely starved and dehydrated people, ridden with typhus and other communicable disease. They did what they could but had to accept their frequent powerlessness to deal with the squalor and to prevent death.

Might the kind of frustration he experienced at Belsen have inclined him away from a career as a practising medic, once he'd qualified? That is certainly possible, but he never recorded such a thought. He expressly *did* abhor the very hierarchical and authoritarian style of many senior hospital medical doctors at that time. Together with his

deep underlying attraction to lab research, his choice to beat his path to the Dunn School becomes understandable.

Florey regarded the principles of pathology to be rooted in experimental physiology. He insisted that new DPhil students who arrived as medics be 'house-trained' by initially undertaking the final year of the Honours undergraduate course in Animal Physiology. Accordingly, Jim studied at Lincoln College, thoroughly relishing the tutorials about the freshest research. He took a First in Finals in 1948, replicating Florey's achievement as a Rhodes Scholar in 1922. Then for his doctorate he investigated whether and how the body's own defences might synergise with the action of certain categories of antibiotics as anti-bacterials *in vivo*. His 165-page thesis, available in the departmental library, additionally describes studies on micrococcin and nisin, two cyclic peptides that were possible candidates to treat *Mycobacterium tuberculosis*. Jim himself fell ill with pulmonary tuberculosis in 1949, forcing convalescence for several months. With his DPhil in the bag in 1952, he then travelled to the Pasteur Institute for one year, in the lab of Pierre Grabar the prominent immunochemist, which seeded a nascent interest in immunology. Upon his return to the Dunn School, the seeds erupted into vigorous germination when Florey suggested he might tackle the still-unsolved "lymphocyte problem". In 1936-1939 Florey had energised his new department partly towards lymphocyte research. What do lymphocytes *do*? Heroic, though unavailing and perhaps ethically questionable, extirpation of all the lymphoid organs of an experimental animal revealed nothing interesting. The nub of the physiological conundrum was the fate of the gigantic numbers of lymphocytes that daily disgorge into the bloodstream from the main lymphatic trunk in the body, the thoracic duct. "*If you can find out where they go, Gowans, you can find out what they do….The lymphocyte problem has blunted the wits of a lot of people in the lab,… and I don't see why you should be spared a similar fate*"[3]. Jim later wholeheartedly acknowledged his indebtedness to Florey: "*It was from Florey that I learned that scientific problems are never solved by polemics but by trying to perform simple, decisive experiments"*.

Between 1954 and 1960, working in room 45[7] which now houses the department's flow cytometry facility, Jim solved the physiological problem. His simple, decisive experiment was to re-infuse a bolus of thoracic duct lymphocytes, collected overnight from a restrained rat, back into its own vein. Count how many flowed from the duct over time, and how many went into the vein. Then do the balance-sheet. He found that the re-infusion prevented the dramatic fall in lymphocyte output from the duct otherwise observed in uninjected controls. Employing radioactive markers[8] to label the injected cells, he proved that the very same cells emerged from the duct a day or so later and revealed the pathway they took. Small lymphocytes therefore comprise a pool that recirculates from lymph to blood to lymph to blood etc in never-ceasing migration. They remain persistent in interphase for extremely long periods: they're not continuously created afresh for a single trip up the duct to be immediately destroyed. They chiefly leave the blood by emigration through the walls of specialised high endothelial venules in secondary lymphoid tissues (though by a different route in spleen).



Brit J Exp Path **38**: 67 (1957)

Apparatus for reinfusing thoracic duct lymphocytes. Made by Harry Stroud, Dunn School workshop, 1955

While Florey was over-optimistic in believing that clarifying the fate of lymphocytes would straightaway reveal their function, Jim's definitive proof of recirculation interwove propitiously with two major strands of immunological thought that were developing fast in the 1950s:

(1) that transplant rejection between unrelated animals is fundamentally an immune response, displaying specific memory and tolerance. Medawar's[9] Nobel-winning work on actively acquired tolerance of foreign cells, with Billingham and Brent in London, was published in 1953.
(2) that specificity is clonally allocated[10]. To deal with the hundreds of millions of possible antigenic threats, one cell is pre-determined by a then-mysterious somatic generator of diversity to exhibit a given epitopic specificity. A clone with a particular specificity required for adaptive immune defence will be exceedingly rare.

Gowans: Bodleian Archive

Jim Gowans (right) with Peter Medawar, in conversation at a reception, probably in 1960

It was Jim, with the co-workers he soon attracted, who showed that pure small lymphocytes *initiate*[11] immune responses, both in transplant rejection[12] and in primary and secondary antibody responses. Cells sleeping in G_0-phase dormancy leap into S-phase hyperaction to become lymphocyte-derived effector and memory cells, of the sort that Florey and many others had often noticed in sites of immune activity. Recirculation of the very large pool of lymphocytes through all the scattered secondary lymphoid tissues solves the "rarity" problem posed by the Clonal Selection Theory. An antigen, wherever it might appear in the body, will be confronted promptly with the right lymphocyte clones to deal with it. He formally demonstrated selection by antigen, notably with Bill Ford, but with others also. His research ramified into mucosal immunology[13], clinical trials of anti-lymphocyte serum to lessen graft rejection, and much else. The story is best told in his own reviews, both contemporary and retrospective[14] and by Irv Weissman[15], Jim's 1964 visitor from Stanford to the department.

Credit: S V Hunt

MRC Cellular Immunology Unit 1969. The whole group is gathered outside the then newly-opened Leslie Martin building, demolished in 2009 to make way for OMPI. Judy Coughlin (nearest camera) sits next to Francis Cooper and Clifford Shayler (animal technicians): Jim Gowans at the back.

In 1963, shortly after he became Henry Dale Professor of the Royal Society, he was invited by the MRC to set up the Cellular Immunology Unit within the Dunn School. His group was never large: it always fitted comfortably within a small coffee-room. Along with the general resources of the Dunn School such as the workshop, it was ably supported by long-serving, very expert and committed technical staff. Judy Coughlin was his indispensable technical assistant, who could cannulate six or more rat thoracic ducts in a day: no problem. She

Photo credit: the late Prof Bent Rolstad

With Jim (smoking his pipe), MRC unit scientists relax at Jim's cottage in Snowdonia, before their assault on the Pyg Track (L to R: Nick Tilney, David Adams, SVH, Bill Ford): 1971.

and the animal technicians in the SPF unit were central to the Unit's success.

The present appreciation has deliberately confined itself to the formative years and how he initially established his international scientific reputation. In 1977 he simply and decisively swivelled away from lab research, discarding his lab notebooks along with thousands of lymphocyte autoradiographs carefully prepared on microscope slides, all heaved into the bin. He became Secretary (CEO) of the MRC for ten years, and after that the first Secretary-General of the Human Frontiers Scientific Programme, until 1993. His job-offer list was extensive, including in 1963 the Chair of Pathology at the Dunn School, prestigious posts in the USA and the headships of several Oxford colleges. He was elected FRS in 1962 and awarded the Royal Medal of the Society; he gained a Gairdner award; a Paul Ehrlich and Ludwig Darmstaedter prize; the Wolf Prize (jointly) and was knighted in 1982. He became closely involved in several medical charities. His own archive in the Bodleian[16], and other obituaries[17] relate these later stages of his career, his interests and the more personal side of his life.

Simon Hunt, DPhil student with Jim Gowans, 1969-72. I'm greatly indebted to Lady Moyra, his widow, and to his three children, Bill, Jenny and Lucy for their help in providing materials for this piece. My deep thanks to Jim for all he did for me are inexpressible in mere words.

Footnotes

1 Mellanby's opinion of Florey
2 Lymph being collected overnight from a thoracic duct cannula has a nasty habit of clotting. The clots need continual removal if you're to have any lymphocytes to study in the morning.
3 From one of his interviews with Max Blythe, 1996-8. The series of five begins at https://doi.org/10.24384/000447
4 Later, he sometimes played for the department cricket team, which used to take on Oxfordshire village teams on their wonderful rural pitches around the county.
5 Frances Allen became her married name: she and Jim retained a life-long friendship
6 Made in 2015 for the Holocaust Memorial Foundation
7 A head-and-shoulders photo of Jim Gowans hangs in the corridor immediately outside room 45, in the "rogues' gallery" alongside those of many of his co-workers
8 ^{32}P-phosphate and ^{3}H-nucleosides had just become available in the early 1950s

9 Jim first met Medawar in 1955, to seek advice how to trace the fate of lymphocytes after transfusions between non-inbred rats . They became very firm friends as well as scientific colleagues. Medawar was himself a Dunn School alumnus (1935-1938) as a young postgraduate, searching for "laws of growth", revealed in cell and tissue explants *in vitro*, under Florey's supervision. In 1937 he married Jean Taylor, a graduate student also in the Dunn School, who worked on Florey's "lymphocyte problem". Was theirs the first match to be made in the Dunn School? It was certainly not the last!
10 An idea, following Talmage and Jerne, that Burnet encapsulated in "The Clonal Selection Theory of Acquired Immunity" (1959).
11 "underwrite" was his favourite word, as though the lymphocyte were an insurance-broker
12 Gowans, Gesner and McGregor (1961) "The Immunological activity of lymphocytes". Ciba Foundation Study Group, page 32
13 He showed, with Julie Knight in 1964, that large lymphocytes from the thoracic duct extensively migrate to the lamina propria of the gut
14 Gowans JL (1968) Harvey lectures 64: 87-119 "Lymphocytes". Gowans JL (1996) Immunology Today 17:288 "The Lymphocyte – a disgraceful gap in medical knowledge": https://doi.org/10.1016/0167-5699(96)80547-0 Fusion #15, Michaelmas 2016,
15 Weissman, IL (2010) Nature Immunology 11:1073.
16 https://archives.bodleian.ox.ac.uk/repositories/2/resources/4328
17 Collected at http://lnnk.in/aahZ, together with his scientific bibliography

Roger Highfield has had an illustrious career in the communication of science, having served as the science Editor of the Daily Telegraph for 20 years and as the Editor of New Scientist from 2008-2011. He is currently the Director of External Affairs at the Science Museum Group and a Visiting Professor at the Dunn School. Roger found time out of his busy schedule to speak to* Fusion *about his passion for science outreach and how scientists can better engage wider interest in their research.

Interview with Roger Highfield

Tell us a little about your role at the Dunn School over the past few years. What has been your impression of the department during the various visits you have made?

Even though I did my degree and doctorate in Oxford back in the 70s and early 80s, it was only in 2017 that I made it a bit further down South Parks Road to the Dunn School, a few yards beyond the old Physical Chemistry Lab where I once did my research on bouncing neutrons. I had been invited to give a talk at a *Future of Science* symposium at the Dunn School and was blown away by the people who were giving the talks, and by the enthusiasm of the graduate students who put the whole event together.

The following year, I was made a Visiting Professor of public engagement – I am hugely indebted to Matthew Freeman, who I have known my entire adult life - and also forged a relationship with Lincoln College, who kindly allowed me to join their SCR. Since then, I have met many Dunn School researchers and have given various talks and masterclasses. When it comes to the latter, on science writing, it made a deep impression: there were a couple of dozen people around the table and it was a brilliantly diverse group, most of whom only spoke English as a second language. They were a talented, enthusiastic lot and it was a wonderful testament to how great science is built on cooperation, collaboration and diversity in all its forms.

What do you feel are the greatest challenges currently facing the department?

The Dunn School shows how in recent years the UK has become a hugely attractive place to do science for young researchers all over the world. Though the government seems to get the point of science (I can remember the time when it was regarded by the Treasury as a cultural activity, like opera), we have had to deal with the huge uncertainty of Brexit. To manage that transition without harming UK science will take a feat of superhuman organisation and, if the handling of COVID-19 at the start of the pandemic is anything to go by, I am nervous.

Thanks to its world-class science, and organisations like the MRC, NIHR and Wellcome Trust, the UK was quick off the mark with COVID-19 research. We launched the world's biggest clinical trials, identified useful drugs, sequenced tens of thousands of virus samples, and have developed the most widely-used vaccine worldwide.
I sincerely hope that, post Brexit, we will have the capacity to do the same when the next pandemic comes along which is inevitable given, for example, that changes in global land use are creating increasing opportunities for spillovers of animal diseases to humans.

What is it that makes you so passionate about the public understanding of science and why do you feel that outreach is so important?

Ultimately, the taxpayer funds a huge amount of research and so we have a moral duty to explain how public money is being spent. As we have seen in the past, for instance when it comes to unease about genetically modified crops, we need the public to be on our side, otherwise we won't get political support and – despite the

Haldane principle – that means we won't get sufficient funding. Perhaps the most remarkable example of how to do it right is reproductive science. From IVF to cloning and mitochondrial donation, the UK has (thanks not least to the late, great Mary Warnock) shown a pragmatic way forward that is the envy of many other countries. That came over clearly when I researched my new book, *The Dance of Life* (co-authored with Magda Zernicka-Goetz).

But there is one thing I want to stress. I don't really aim to *educate* the public (so I don't use the phrase 'public understanding of science') for two reasons. The 'public' is a meaningless term, since a molecular biologist is as ignorant about particle physics as a particle physicist is about molecular biology as a three-year-old is about either of these fields of research. And, at the Science Museum, we are trying to start a conversation with target audiences about science, not lecture them.

What are some of the common mistakes that scientists tend to make when presenting their research to a non-specialist audience?
At the Dunn School masterclass – like many other encounters with young academics, for instance the MRC's Max Perutz science writing prize - I was struck by how researchers think the core issue is to help the public understand what they are doing. They were keen to explain how science worked, the details of their research.

But they are mistaken. The real challenge is to earn the right to explain arcane science. That means thinking of a hook or angle to get your intended audience interested in the first place, so they ask you to tell them more. If you don't get that right, you come over as a bit of a bore. Who wants an explanation of something they are not interested in?

How do you sense the Dunn School is currently viewed by the public and how might we alter this perception for the better?
The Dunn School's unique selling point is fundamental biology that underlies human disease, a mission with universal appeal. However, as I have said, 'the public' is a patchwork of different audiences and I think the reputation of the Dunn School will depend which one you are talking about – other researchers or politicians or kids, for example.

The Dunn School's place in history is, of course, assured so that will count a lot to many people and I think it was great that it celebrated its pivotal role in the development of *the* wonder drug of the 20th century with a blue plaque. Many know the story of how Alexander Fleming recognised the potential of penicillin. But it took critical research at the Dunn School for the revolution to truly begin thanks to Ernst Chain and Howard Florey, who recruited the 'penicillin girls' (Ruth Callow, Claire Inayat, Betty Cooke, Peggy Gardner, Megan Lancaster and Patricia McKegney) to farm the drug, and of course Norman Heatley, known by some as 'the forgotten man of penicillin', who fashioned a purification system out of a bookcase in the face of wartime shortages. Other key players in the rise of antibiotics include another Oxford Nobelist, Dorothy Hodgkin, who solved the molecular structure of penicillin. We have lots of her models in our collection of seven million things, notably penicillin. It is a great story.

Looking forward, when it comes to improving the perception of the Dunn School, it is important to start with the fundamentals. Yes, you can always come up with glossier PR, slick spokespeople (Matthew Freeman is very accomplished!) but, ultimately, you have to do great science and by great I mean research that fundamentally changes the way we look at the world, or that has impact in years to come, whether in drugs, vaccines, diagnostics or whatever.

Ultimately, it is all about people. Maintaining a lifeblood of motivated, talented, curious people is the most important and meaningful indicator of future success because the best young group leaders (and postdocs and students) are in such great demand. We need to recruit the very best young scientists to work at the Dunn School - and we can!

Scientists are consistently urged to commercialise their research. In this edition of* Fusion*, we hear first from Neil Barclay about his success in commercialising antibodies as research reagents, interview Ben Dodsworth about the start-up inspired by his time in the department, and find out more about OXvax, the latest Dunn School spin out.

Climbing Mount Everest: Commercialisation of Antibodies as Research Reagents

Neil Barclay

Commercialisation of monoclonal antibodies for research
In the early days of immunology research, a starting point was often to make an antibody in order to be able to study proteins. This became much easier once it was possible to make monoclonal antibodies from 1976 onwards. Alan Williams had started making monoclonal antibodies in the Dunn School in 1978 and several had been made by the time I arrived later that year. They were very popular, so it soon became a problem to produce sufficient volumes of antibody to supply other researchers requesting them. I was involved in setting up commercialisation of these reagents through

companies such as Serotec (now BioRad) with the Medical Research Council handling the licensing. The main aims were to ensure that the quality was first class and the antibodies were readily available. We did not see this is a revenue-earning venture although the small amounts of royalties were useful in the laboratory. Indeed all royalties went to the laboratory fund except for a charge by the MRC.

The mid 1980's saw a change in culture in that researchers were much more willing to buy reagents rather than make them in-house – a change driven by the introduction of recombinant DNA technology – and royalties steadily increased. In 2010 we were able to set up a charitable trust to manage the funds obtained from royalties in an efficient way – the CIU Trust. It also allows funds to be donated from other sources and designated for particular aims. For instance, it collected funds in memory of James Porterfield, an eminent virologist at the Dunn School, that were then used to help young virologists to visit other laboratories.

A need for polyclonal antibodies? Everest Biotech.

In 1999 my DPhil student Nick Hutchings and I were looking to identify proteins associated with the cytoplasmic regions of cell surface proteins. Our first thought on finding a band on a gel was to make an antibody but with the genome recently sequenced what we decided we really needed was to have antibodies available against everything. Nick had friends from Nepal and came back the next day saying why not make antibodies against *all* known proteins – there were 6 million goats in Nepal and we could make anti-peptide antibodies. It seemed a crazy idea but one that might be feasible. In 1999 Nick did a pilot study in Nepal that proved to be successful and so we began trying to find investors. The sources of venture capital we tried around Oxford were not interested in investing in a factory in Nepal. There was, of course, no intellectual property. However, after about 9 months we found a business angel, Garf Collins, who was attracted to the idea and the company started trading in 2000. It went through many challenges because of its environment such as the Maoist uprising, the massacre of the Nepalese royal family and a major earthquake. However, it made more than 3000 reagents in Kathmandu, all affinity purified using the immunising peptide. The operation in Kathmandu was spun out and a new company called Shikhar Biotech established which still provides antibodies, despite the main company being taken over in 2019 by another reagent company, LSBio Ltd.

Recombinant antibodies – the gold standard. Absolute Antibody Ltd.

Although polyclonal antibodies have advantages, they are, nevertheless, limited. They are difficult to reproduce and quality control. Even monoclonal antibodies are not completely 'monoclonal' as they can contain other immunoglobulin light and heavy chains. The ideal reagent is a recombinant antibody. The establishment of the technology of making recombinant antibodies for therapeutic purposes provides a way to make chemically-defined antibodies that can be used as reagents for research purposes. For this, the coding sequence for the antibody is needed and this is usually obtained by sequencing the cDNA from the monoclonal cell line. A construct is prepared, and the protein expressed in a cell line such as Chinese Hamster Ovary (CHO) cells or human embryonic kidney lines which are suitable for producing large amounts of proteins. In 2019 Absolute Antibody made over 3000 antibodies amounting to 120 grams of antibody in total. Recently it has been making antibodies and other recombinant proteins for the COVID-19 research effort. One of the advantages of the technology is that one can readily make variants with different potential effector functions by changing the Fc regions.

As shown above, commercialisation leads to more commercialisation and Absolute Antibody has recently spun out another company called mAbSolve under the direction of Geoff Hale. Geoff was involved with Herman Waldmann in the very early work on CAMPATH-1, one of the first therapeutic antibodies ever made, and has been instrumental in developing and commercialising immunoassays through BioAnalab, Absolute Antibody and now also mAbSolve as well as another company, Native Antigen Ltd, which is also heavily involved in COVID-19 research.

Conclusions

Biomedical research is heavily dependent on good reagents. Unfortunately, those reagents that are available are not always of the highest quality. The move to recombinant antibodies with full citation of their use, should be a step in the right direction. The link between academia and industry can play an important role in this and industry can be a powerful partner to aid research, particularly in times of crisis, such as the Covid-19 pandemic.

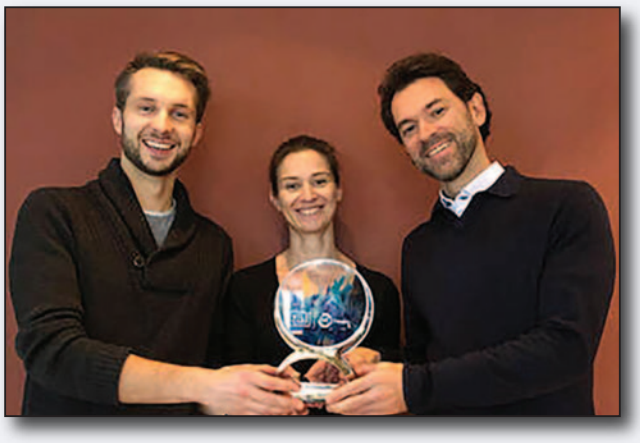
Everest Biotech advertised at the top of Mount Everest through our corporate sponsorship of Child Rescue Nepal that helps Nepal's exploited children.

After completing a DPhil and post-doctoral study at the Dunn School, Ben Dodsworth honed his entreprenerial skills to establish PIPRA, a start-up focussed on post-operative delirium, a specific unmet medical need. **Fusion** *caught up with him to find out about the highs and lows of his new venture.*

Interview with Ben Dodsworth

What is your association with the Dunn School and for how long were you a member of the department?
I was a member of the department for 4.5 years - firstly as a DPhil student between 2014 and 2018 and then as a postdoc from 2018 to 2019.

What inspired you to enter the EIT Health Wild Card New Ventures competition and what did the process involve?
I was searching for something on the intersection of three key elements I enjoy: science, impact and entrepreneurship. The wildcard competition was perfect for that. I went into it with a couple of ideas myself, but also open to joining a great team with a big idea.
The first stage is a week-long hackathon designed to bring teams together. This is how I met my co-founders John (business lead) and Nayeli (medical doctor and anaesthetist). During this week, we were coached, challenged, and finally selected for the next round – a ten-week accelerator programme. At the end of this second round, we pitched for two million Euros of investment in possibly the most intense hour of my life. And we won! (Figure 1).



Tell us a little about PIPRA and its mission
We're working on post-operative delirium (POD) which affects a staggering 30% of patients over sixty after surgery. Symptoms include disorientation, memory loss and difficulties in speech. However, the secondary adverse outcomes are much more severe: 25% mortality within one month, double the risk of nursing home admission, 38% will suffer long-term cognitive decline and dementia. I've spoken to over 150 different patients, relatives and clinicians about POD and one particular story stuck with me. A grandfather used a wheelchair due to problems with his knee. He was eager to play with his grandkids in the garden again, and therefore his GP recommended surgery. Nobody informed him about the risk of cognitive decline. After surgery, he developed POD. He never recognised his grandchildren again. Instead, he spent the remaining years of his life in a nursing home. I doubt he would have chosen surgery if he had known about how it could turn out - but right now, there is no formal way to quantify that risk or get it into surgical decision making. Experiences like these drive us to do more and move faster. We need to improve these odds so we can all live long, healthy, and happy lives.

Right now, there are no treatments for POD available once symptoms arise. Instead, the focus is on prevention. Many highly effective preventative treatments have been developed but are too costly to deploy for every patient. So, at PIPRA, we're addressing this unmet need by developing a novel, AI-based pre-operative risk prediction tool which highlights patients at risk before undergoing surgery.

There is some good news. We were worried about clinical awareness of POD since patients who have cognitive decline can quickly drop out of the system and not be recorded. In contrast, a patient who has an obvious complication, such as pain, will come back to the hospital and will influence the statistics. However, hospital follow-up has improved dramatically over the last decade, and in a survey with over 120 anaesthetists, we found only two who were not aware of the issue.

Finally, I think the quickest vehicle to make a sustainable improvement of the standard of care is a start-up. You can move incredibly fast, and you have scalability at the core.

How have you found the move to cognitive impairment and AI?
Fascinating! I find it bizarre how the most effective preventative measures seemingly have nothing to do with the pathophysiology. The current dogma is that systemic inflammation from a surgical insult leads to neuroinflammation. This, in combination with microemboli (essentially mini-strokes), causes post-operative delirium. There's overwhelming evidence that a bundle of simple nursing measures massively reduces the incidence of post-operative delirium (by approximately 40%!). These measures include: a nurse being with the patient when they wake up, telling the patient where they are and why, placing them near a window so they have a feel for the time, making sure the patient wears their glasses and hearing aids when they wake up and regularly checking that they are not dehydrated. How does this fit with neuroinflammation? Not a clue but the stats and the numbers are striking. To me, this is a strange but delightfully practical approach.

Moving into the field of AI has also been fun. I'm no AI expert (yet!), but I'm thoroughly enjoying the kind of problems we are solving.

What have been some of the highs and lows of establishing a start-up company?
Incredible highs and so many lows, so I've sketched them (below). The most recent and maybe the most spectacular high to low to high was our clinical trial. We received ethics approval in record time, and it was due to start in March 2020. Then the Coronavirus hit, and we had to cancel it. However, shortly after, we received incredible data from other sources, and then the EU decided to postpone the Medical Devices Regulation. This means that the clinical trial was not required after all, and we can reach the market faster and more cost-effectively.

How did your training at the Dunn School prepare you for the challenges you have faced?
The Dunn School is a spectacular environment, and a DPhil is a great way to learn the right kind of skills. Of course, it is possible to focus entirely on learning western blots and PCR, but there is much more that I picked up on the way or explicitly sought out. For example, how do you collaborate? How do you find and recruit the best people to work with you on your project? How do you reward collaborators? How do you conduct yourself during conferences? How do you find the balance between self-motivation and keeping a sustainable work-life balance? The Dunn School has created an environment which is great for learning these skills. You have excellent seminars and great work by the GSA who, at the time I spent at the Dunn School, was pushing to show a bit more career diversity. I think that, for the challenges I have faced, the most crucial skill the Dunn School helped me develop was resourcefulness.

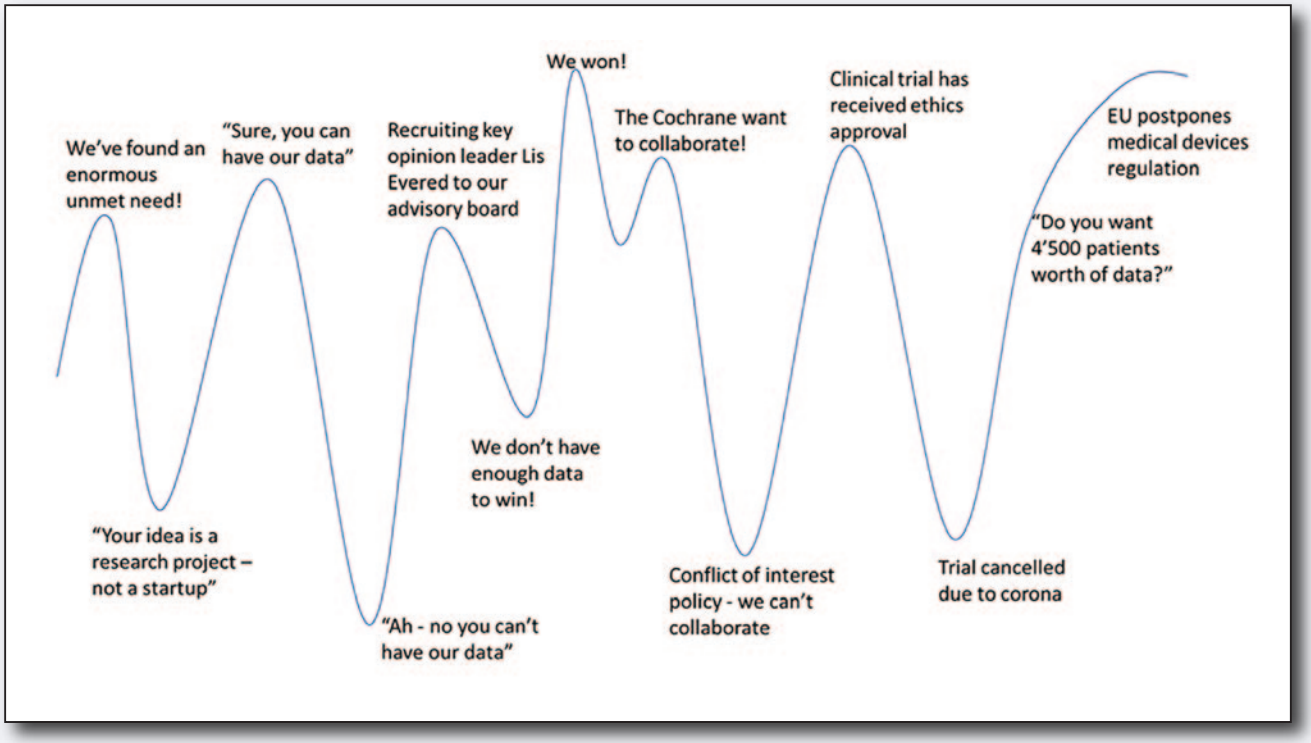
How do you see your career developing in the future?
Your guess is as good as mine. We are raising further capital in Q3 2021 – that will determine where we're heading!

What advice would you give other budding entrepreneurs who might be tempted to follow your example?
Make the most of the Oxford ecosystem! The university has an incredible number of events and courses that you can attend. I benefited from Ideas2Impact led by the Saïd Business School, Navigator (or Springboard) and many other skill-based workshops (presentation skills etc.). I highly recommend looking at what MSD, MPLS and the careers service have on offer. A DPhil is a chance to build up your skills, and the university has a tremendous amount of expertise, so if you need advice about anything, there is usually someone who can help. For example, I was offered equity in a start-up in exchange for my time – however, I had no idea how much was reasonable. Since this was a highly specific question, the careers service was able to put me in touch with one of their specialists who does not usually make appointments. If in doubt, it is worth asking!

Finally, two pieces of advice which were given to me and I found particularly useful:

1. You cannot know everything – find co-founders with complementary skill sets. If you are unsure about how to do that, there are special programmes designed to make those connections.

2. If you have a brilliant idea, do not hold on to it too tightly. Focus on the problem, rather than the solution. And if you don't have an idea – no worries. You can bring your skills to an existing one.



OXvax: Exploiting the Properties of Stem Cells for Cancer Immunotherapy

Paul Fairchild

Harnessing the power of the immune system to eradicate cancerous cells is a concept that is far from new. Indeed, numerous strategies now exist that recruit the firepower of the T cell repertoire or endogenous populations of Natural Killer cells to target a solid tumour. While many successes have been reported, the range of cancers that have so far benefitted is extremely limited. Other approaches have sought to use monoclonal antibodies specific for inhibitory receptors to interfere with the natural breaks of the immune system, thereby amplifying the magnitude of the immune response. But so-called immune checkpoint inhibitors (ICIs) have their own limitations: they are, for instance, only effective where an immune response to tumour antigens has been initiated which is not always the case, given that tumour antigens are self-components that would not normally pose a threat. Additional immune intervention may, therefore, be required to ensure that an immune response is first established that ICIs can subsequently amplify. It is against this background that there has been a recent resurgence of interest in the use of dendritic cells as a natural way of guiding the immune system to mount a proportionate response to a tumour.

Dendritic cells: Choreographers of the immune system

Dendritic cells are attractive candidates for immunotherapy since they are responsible for accurately deploying the immune system in response to an existing threat; indeed, all immune responses, whether protective or pathogenic, are first initiated at the surface of dendritic cells as they show fragments of antigen to T cells, setting them on the trail of a potential killer, like bloodhounds given the scent of a fugitive. But not all dendritic cells were created equal, indeed, the diversity of dendritic cell subsets has become progressively clear over recent years which appears to permit division of labour when mounting an immune response. While so-called 'plasmacytoid' dendritic cells are optimally adapted for sensing viral infection and secreting interferons by way of a response, a subset defined by its expression of the cell surface protein CD141 displays the capacity for 'cross-presentation' of antigenic fragments from exogenous antigens direct to cytotoxic T cells. It is this property that most other subsets lack, favouring, instead, the presentation of antigenic fragments to helper T cells to facilitate antibody production, the most effective defence against bacterial infection. How to tap into this natural resource in order to elicit an immune response to an established tumour that is both appropriate and proportionate has proven to be something of a challenge over the past decade.

Past experiences in the clinical use of dendritic cells

The role played by dendritic cells in eliciting immune responses has inspired many attempts to harness their properties for the purpose of cancer immunotherapy, but success has been severely hampered by the issues of access and availability. While subsets such as the $CD141^+$ population would appear highly adapted to invoke the cytotoxic T cell responses required to target an established tumour, it has proven impossible to access sufficient numbers of cells from patients for effective immune intervention: even an entire apheresis of a patient yields only a few hundred thousand cells, woefully inadequate for a single dose! It is for this reason that most clinical trials to date have focussed on the use of circulating monocytes from patients that are abundant in peripheral blood and can be differentiated *in vitro* into immunostimulatory dendritic cells. But what such an approach gains in terms of accessibility, it loses in efficacy since monocyte-derived dendritic cells fail to emulate the properties of the $CD141^+$ subset, in particular their capacity for antigen cross-presentation direct to the cytotoxic T cell repertoire. Indeed, it is this factor alone that may explain the disappointing outcomes of over 200 clinical trials for the treatment of numerous malignancies from melanoma to prostate cancer and from glioblastoma to renal cell carcinoma. It was largely in response to this impasse that the Fairchild laboratory set out, some years ago, to investigate alternative sources of dendritic cells that might overcome many of the current limitations.

Pluripotent stem cells: The pathway to abundance?

Pluripotent stem cells are, by definition, capable of differentiating into any one of the cell types that make up the mammalian body and have the significant advantage that they can be maintained indefinitely *in vitro* and scaled up over time to produce almost limitless numbers of differentiated progeny. We therefore investigated whether embryonic stem cells (ESC) - the quintessential pluripotent stem cells - might provide an alternative source of dendritic cells for clinical applications. Indeed, we were the first to decipher the differentiation pathway of mouse ESC into dendritic cells[1] (Figure 1) which we subsequently adapted for use with ESC of human origin[2]. The patent we filed on our technology was licensed by Geron Corporation and subsequently Asterias Biotherapeutics who have since made use of our protocols in clinical trials for the treatment of non-small cell lung cancer with promising early results. However, this novel source of dendritic cells turns out to harbour the same limitations as monocyte-derived dendritic cells, namely their inefficiency at antigen cross-presentation, raising the question of

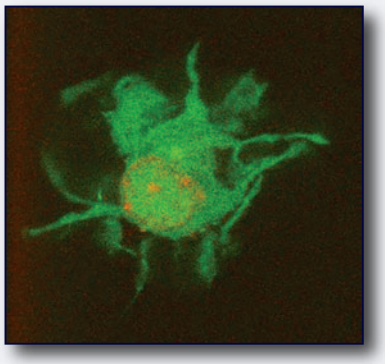


Figure 1 A single dendritic cell, labelled with green fluorescent protein, differentiated from mouse ESC.

whether even better results would have been obtained had CD141$^+$ dendritic cells been use instead.

To address this question, we therefore returned to the drawing board and made three critical changes to our original protocols: firstly, we dispensed with ESC, which carry significant ethical baggage, choosing to make use of so-called induced pluripotent stem cells (iPSC). These can be derived from any somatic cells of the body by the introduction of as few as three transcription factors know to govern pluripotency, a discovery that earned Shinya Yamanaka the 2012 Nobel Prize for Physiology or Medicine (Figure 2). Critically, this breakthrough allows the derivation of pluripotent stem cells direct from individual patients, something that could never be achieved using ESC.

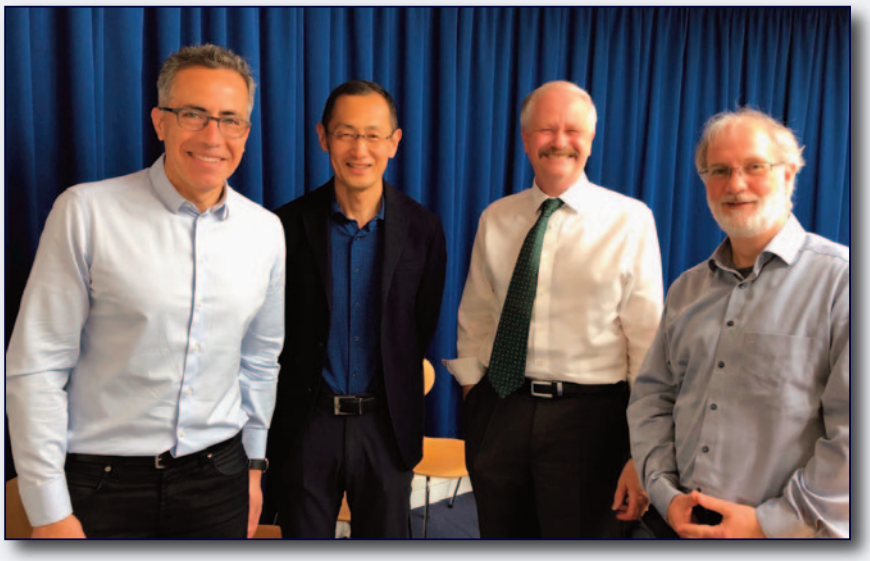


Figure 2. The co-founders of OXvax meet with Shinya Yamanaka during his visit to Oxford to deliver the 2019 Jenkinson Memorial Lecture. (Left to right: Marcelo Bravo, Shinya Yamanaka, Paul Fairchild, Tim Davies).

Secondly, we modified our existing protocols for the differentiation of a specific subset of dendritic cells - those defined by expression of CD141 - that would be most likely to elicit the kind of response required to target a solid tumour[3,4]. And thirdly, we introduced subtle changes to the way we derive our iPSC, including the starting cell type we obtain from patients, so as to ensure that dendritic cells differentiated from them are highly immunogenic[5], capable of stirring a dramatic response among resting cytotoxic T cells. The patents we filed as a result of this work therefore provided the potential to obtain an abundant source of CD141$^+$ dendritic cells for clinical applications, something that had never previously been feasible, forming a strong basis for a spin out company focussed on cell therapies for cancer treatment. A chance meeting in 2017 with Marcelo Bravo, a serial entrepreneur based in Oxford, provided the necessary commercial insight and expertise to begin shaping a proposition to take to potential investors.

The launch of OXvax

The pathway to securing investment turned out to be long and at times arduous with many unexpected challenges along the way: the untimely arrival of Brexit disrupted essential supply chains, threatening to scupper the *in vivo* experiments we needed to strengthen our proposition; the fallout from the collapse of local, high-profile equity funds left potential investors uneasy and risk averse, while the unwelcome arrival of the pandemic had a devastating impact on our ability to meet with investors and foster critical relationships. Nevertheless, seemingly against all the odds, we were able to secure seed funding for the venture from two sources: Lead Compass, a venture capital fund based in South Korea and Evotec, a German drug discovery and development company. With such strong backing, OXvax launched in April 2021 and will be based in laboratory facilities at the Bioescalator on the Churchill Hospital site. The initial focus of the company will be to define the quality profile of our cell therapy product and the industrialisation of the manufacturing protocol, in order to work towards first-in-man clinical trials for the treatment of solid tumours. While Marcelo Bravo assumes the role of CEO, Tim Davies, who has been instrumental in developing the technology and who is a co-founder of the company, will become Head of Laboratory Operations. They will be joined by new recruits, Nicole Bedke as Head of Immunology and Kate Silk, a former post-doc in the Fairchild laboratory, to form a team with the necessary skills to meet early milestones on the pathway to the clinic.

Acknowledgements

It is, of course, not possible to write about OXvax without acknowledging the many sources of support we have received along the way, most especially from the Dunn School. We have received financial backing from the EPA Trust and the Guy Newton Translational Fund, without which we would never have been able to acquire the necessary preclinical data that investors required. We are especially grateful to Matthew Freeman for the many and varied ways in which he has helped facilitate the process, providing both practical and moral support over the past few years. However, we are also indebted to the guidance provided by Oxford University Innovation (OUI) and, in particular, to our program managers Richard Reschen, a Dunn School alumnus, and Andrew Chan, both of whom worked tirelessly to negotiate with investors and close the final deal. But on a personal note, I would also like to pay tribute to our former Head of Department, Herman Waldmann, with whom I had the honour of working for many years. Herman was not only instrumental in the early stages of the work that has culminated in OXvax, but first introduced me to translational research and the pathway to commercialisation: his boundless energy and passion for science will always be an inspiration!

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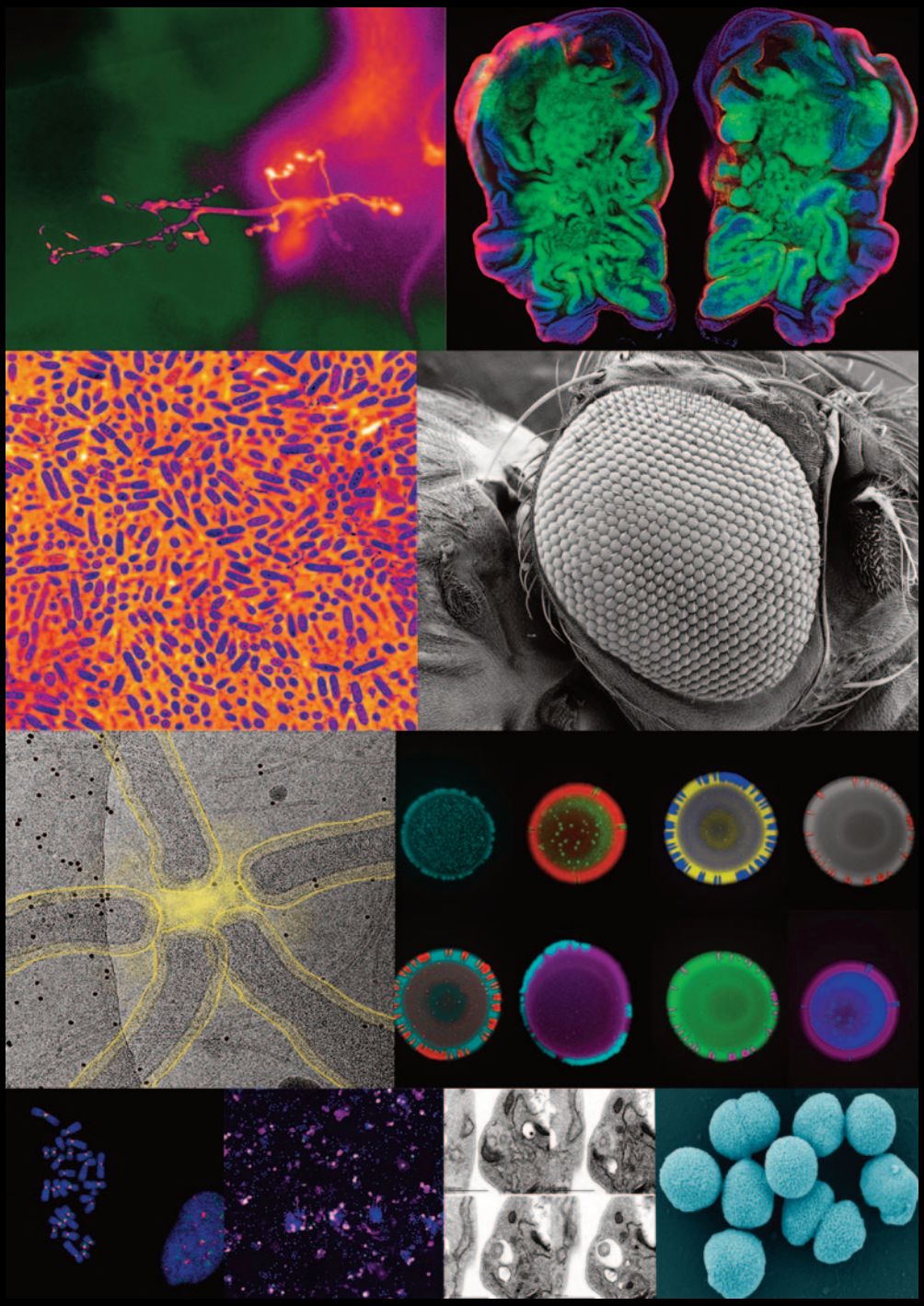
Dunn School Bioimaging Facility Image Awards 2019



Top left: Franz Wendler (Baena lab): 'In the heat of the moment'. Confocal image of the neuromusular junction at muscles 6,7 in *Drosophila* 3rd instar larvae.

Top right: Derek Xu (Baena lab): 'Pseudo cerebral'. Confocal image showing separate focal planes of a tumorous *Drosophila* wing imaginal disc, reflected about a vertical axis.

2nd row left: Charlotte Melia (Bharat lab): 'Pseudo-coloured Pseudos'. False coloured TEM image of an ultrathin resin section through a *Pseudomonas aeruginosa* biofilm.

2nd row right: Sonia Muliyil (Freeman lab) and Faith Kim (Carvalho lab): 'The Fly'. An SEM image of *Drosophila*.

3rd row left: Nina Sulkowski (Bharat lab): 'Caulobacter Christmas Star'. False coloured cryo-TEM image of *Caulobacter crescentus*.

3rd row right: Rafael Da Silva Custodio (Tang lab): 'Cosmic warfare'. The spatial distribution of different strains of fluorescently-labelled *Neisseria* competing for space and nutrients on solid media.

Bottom left: Emily Graham (Ahel lab): Centromeric COFISH (chromosome orientation in situ hybridisation) image.

Bottom centre left: Claire Hill (Alberto Baena lab): Stitched fluorescence image of *Drosophila* cells.

Bottom centre right: Ciaran McCoy (Gluenz lab): TEM of serial sections through the base of a *Leishmania* flagellar pocket.

Bottom right: Edeline Yee (Tang lab): A false-coloured SEM image of *Neisseria gonorrhoeae*.

SPOTLIGHT

Autophagy and (+) RNA Viruses – An Intricate Relationship

Sophie van Leur and Sumana Sanyal

Autophagy is an evolutionarily conserved central process in host metabolism. It functions to conserve energy during starvation, recycle organelles and degrade long-lived proteins. Furthermore, autophagy represents a primordial intrinsic cellular defence mechanism and plays a critical role in removing intracellular pathogens. Not surprisingly many pathogens have co-evolved strategies to manipulate this pathway and use it to their advantage. Particularly intriguing is the relationship between autophagy and positive sense (+) RNA viruses. Our research group focuses on how flaviviruses such as Dengue and Zika benefit from this pathway without succumbing to lysosomal degradation.

What is autophagy?

Christian de Duve first coined the expression 'autophagy' during his seminal work on the discovery of lysosomes, for which he was awarded the Nobel Prize in Physiology or Medicine in 1974. Later, genetic screens performed in yeast by Yoshinori Ohsumi revealed at least 15 genes that were involved in autophagy, many of which had orthologs in humans. For his work on the genetic basis of autophagy, Yoshinori Ohsumi was awarded the Nobel Prize in 2016.

Autophagy is initiated by sequestration of cytoplasmic proteins and damaged organelles into crescent-shaped double-membrane vesicles. Once contents are captured, the immature isolation membranes expand to form autophagosomes, which subsequently fuse with lysosomes, thus forming autolysosomes. The contents undergo degradation within the autolysosomes to enable recycling during starvation. Approximately 40 genes have been reported to date which participate in the process of autophagosomal degradation.

Autophagy and (+) RNA viruses

A link between autophagosomes and virus-induced vesicles was first proposed by George Palade using EM imaging of poliovirus-containing vesicles that resembled autophagosomal membranes[1]. Over the past few decades, a growing body of research has defined the pro- and antiviral effects of this pathway in the lifecycle of numerous (+) RNA viruses. Our own research has shed light specifically on the critical role autophagy plays in driving the intracellular assembly and secretion of Dengue and Zika virus particles.

Selective autophagy for the assembly of virus progenies

Apart from the relatively non-specific bulk macro-autophagy, cellular organelles are turned over through several types of selective autophagy. This phenomenon occurs under normal physiological conditions and is hypothesized to appropriately address a specific stress. During Dengue/Zika virus infection, selective autophagy of lipid storage organelles (lipid droplets) - commonly referred to as lipophagy - hydrolyses neutral fat deposits to generate free fatty acids and cholesterol, resulting in rapid loss of lipid droplets within the population of infected cells[2,3]. Our subsequent study revealed that lipophagy supported assembly of viral progenies by initiating biogenesis of assembly sites via the acyltransferase activity of AUP1, a type-III membrane protein with dual localization between the endoplasmic reticulum and lipid droplets. AUP1 was found to be regulated by multiple monoubiquitin modifications. Either infection or a combined expression of the viral NS4A and NS4B proteins were necessary and sufficient to generate the unmodified form of AUP1, a step that was critical in induction of lipophagy[2] (Figure 1).

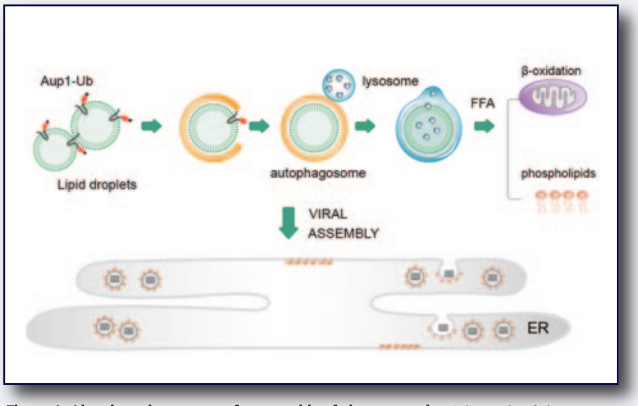


Figure 1. Lipophagy is necessary for assembly of virus progenies Schematic of the mechanism of AUP1-dependent lipophagy in Dengue virus infected cells. Monoubiquitin-modified AUP1 appears in its unmodified form in lipid droplets upon infection. The acyltransferase enzymatic function is active in unmodified AUP1, which results in hydrolysis of lipid droplets to generate free fatty acids. These, in turn, undergo β-oxidation and produce the necessary phospholipids to collectively generate the viral assembly sites.

Non-lytic viral transmission by secretory autophagy

Apart from the degradative role of autophagy, more recent studies have highlighted its non-degradative role in secretion. Several reports on mechanisms of cell-to-cell transfer of intracellular pathogens indicate non-degradative autophagic vesicles as an efficient mode of transport. Secretory autophagy is a newly-discovered pathway in which autophagosomes fuse with the plasma membrane instead of lysosomes and release single membrane vesicles containing cytosolic content into the extracellular milieu[4].

Non-degradative autophagy has been suggested to facilitate non-lytic egress of some (+) RNA viruses. The initial characterisation was with enteroviruses, which appear to exploit this pathway to exit cells, and are released into the extracellular environment as particle populations contained within vesicles. Clusters of enteroviral particles are packaged with phosphatidyl serine into autophagic vesicles, which enable efficient transfer to macrophages, significantly enhancing viral infectivity. This revealed a novel mode of transport where viral genomes were transferred *en bloc* to recipient cells, facilitating genetic cooperativity and enhancing infection[5].

Our recent work has revealed that a similar strategy is used by Dengue virus too. Extracellular viral progenies from infected hepatocytes were found in distinct populations, one as free particles and the other within

autophagosome-derived vesicles, where release of the latter was regulated by Lyn kinase[6] (Figure 2). Secretory autophagy has also been implicated in vertical transmission of Zika virus. Autophagic activity in human trophoblasts restricted by pharmacological inhibition, or by deficiency of an essential autophagy gene, Atg16l1, limited Zika vertical transmission and improved placental and fetal outcomes, which supported a role for autophagic secretion in the process.

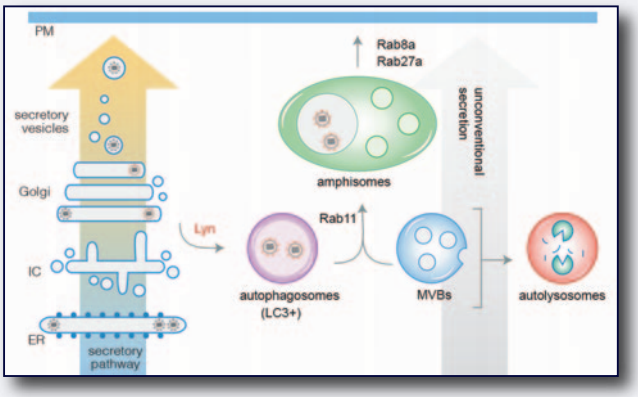


Figure 2. Lipophagy is necessary for assembly of virus progenies Schematic of the model of unconventional secretion of virus particles. Mature Dengue virus progenies trigger biogenesis of specialised secretory organelles derived from autophagosomes that facilitate efficient secretion. These $LC3^+$ organelles are either secretory autophagosomes or amphisomes (generated upon fusion of the autophagosomes with multivesicular bodies or late endosomes) that in the absence of Lyn, fuse with the lysosomal compartments for degradation. Immature virions, on the other hand, are unable to trigger this pathway and are instead exported through bulk exocytosis, as illustrated.

Perspectives

Viral exploitation of autophagic vesicles for delivery to the extracellular medium implies that these viruses could evade neutralising antibodies and increase viral spread. Double staining of Dengue antigen and LC3 and their sensitivity to antibodies or detergent in a close-contact co-culture experimental set-up, support this hypothesis[6]. An interesting question that arises from the induction of autophagy in the context of Dengue infection, is the process of antigen presentation. Monocytes and monocyte-derived cells are a major target of flaviviruses, where antigen presentation is facilitated by autophagy. This implies that while autophagy favours production of viral progenies, it should simultaneously increase viral antigen presentation and T-cell responses, thus promoting cytotoxic T-cell killing. Information on these seemingly contradictory processes is currently limited. However, Dengue-infected human monocyte-derived cells fail to upregulate MHC and co-stimulatory molecules and have an impaired ability to polarize $CD4^+$ Th type 1 effector properties, contributing to inefficient adaptive immune responses observed in patients. In bulk cultures of dendritic cells, exposure to Dengue augments MHC class I and MHC class II expression in non-infected bystander cells, however, the infected population of monocyte-derived dendritic cells displays an inhibition in this process within the same cultures. In clinical studies, gene expression analyses of Dengue-infected patients revealed that severe cases expressed lower levels of genes linked to antigen processing, presentation and T-cell activation compared to mild cases. Thus, impaired antigen presentation and functionality of virus-infected cells may reflect a viral immune evasion strategy to dampen T cell responses and impact disease severity. One possibility is that Dengue activates autophagy only during the early stages of the viral lifecycle, suggesting a biphasic response of autophagy to infection, where it shifts from a supporting to an antiviral role at later time points. Further studies will enable us to reconcile how flaviviruses have evolved strategies to manipulate this pathway while subverting T cell-based immune responses.

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RESEARCH INSIGHT

Vaccine Development: Successes, Challenges and Prospects

Christoph Tang

Vaccines have made a huge contribution to human health across the world. Jenner's pioneering studies on Smallpox in the late 18th century resulted in the use of vaccinia immunisation across Europe within two decades and the eventual declaration by the World Health Assembly in 1980 that smallpox had been eradicated. The WHO Expanded Programme of Immunization (EPI) was formed in 1974 to ensure that vaccines reach those at most need, particularly children in less wealthy countries. The EPI programme was subsequently strengthened by the Global Alliance for Vaccination and Immunization (GAVI). As a result, polio virus has nearly disappeared from the face of the planet, and steps are being taken to target measles virus for elimination. Most recently, we have witnessed the remarkably rapid development and deployment of vaccines against Covid-19, with mRNA-based vaccines being the breakthrough technology of 2020.

Despite these triumphs, challenges still abound for the development of vaccines. First, vaccines are a victim of their own success. With a vaccine programme in place, society can readily evade the ravages of diseases such as smallpox and diphtheria, however, vaccines that are administered as part of national programmes are given to large numbers of healthy people, so safety is a major concern. When given at such a scale, even rare side effects result in a significant number of

cases and sometimes adverse publicity. This concern has led to the shift from vaccines based on complex preparations (such as live attenuated or killed organisms), to highly engineered so-called 'sub-unit' vaccines. Second, aside from emerging infections such as Covid-19 (against which many vaccines have proven effective), it is widely considered that vaccines have already been made against most of the 'easy targets'. For example, when disease is caused by a single molecule, such as the diphtheria or tetanus toxins, immune responses directed solely at these molecules are highly effective and can prevent cases. Third, whole genome sequencing (WGS) has revealed the full extent of diversity of antigens expressed by many pathogens, including *Neisseria* and *Staphylococcus aureus*. Additionally, host adapted microbes have evolved sophisticated mechanisms, such as assuming an intracellular lifestyle, which can subvert immune responses during infection and/or induced by vaccines. Finally, now that many childhood infections have been prevented, there has been a shift to developing prophylactic vaccines to protect vulnerable individuals (including the elderly and immunocompromised) whose immune responses to vaccine antigens are often suboptimal.

So what are the prospects given these challenges? Remarkably, more progress has been made developing vaccines compared with antimicrobial drugs, despite the need for new antibiotics given the emergence of antimicrobial resistance (AMR) in many important bacterial pathogens. Advances have been achieved through biological insights as well as technical innovations, with some of the challenges actually turned into advantages. For example, while data from WGS has provided an unprecedented view of the diversity of microbes, the information can be exploited for vaccine design; WGS contains information about every potential vaccine antigen that could be expressed by a pathogen. Understanding host-pathogen interactions has identified many host evasion and colonisation factors. This knowledge has been applied to the search for new immunogens. For example, conjugate vaccines against the pneumococcus include the bacterium's capsule, which prevents its phagocytosis by macrophages. These vaccines are given to children worldwide through the EPI and generate sales of over £3 billion annually. For this reason, it is proposed that vaccines will be invaluable for tackling the growing threat of AMR in pathogens.

My group works on defining the mechanisms employed by human adapted bacterial pathogens to colonise and cause disease. Given the long term and intimate relationship between this class of bacteria with their host, we aim to unravel the specific adaptations that enable them to survive within niches found in the human body and their strategies to avoid immune responses. The longer-term aim of the group is to translate our findings into approaches to prevent or treat infections caused by these bacteria, with part of the group focussed on the two pathogenic members of the genus *Neisseria*, *Neisseria meningitidis* and *Neisseria gonorrhoeae*.

N. meningitidis is a leading cause of rapid onset septicaemia and bacterial meningitis in children and young adults. This bacterium frequently causes asymptomatic carriage; typically, around 10% of young adults are colonised with carriage of individual strains lasting for several months. Therefore, the bacterium can successfully evade the immune response while resident in the upper airway. Virulent strains of the bacterium express a polysaccharide capsule on their surface, belonging to serogroups A, B, C, Y or W. Aside from differences in capsule expression, *N. meningitidis* is a highly diverse species, in part because it can undergo horizontal gene transfer and can acquire DNA from the environment, allowing rapid genetic change.

There are effective vaccines that elicit immune responses against the A, C, Y and W capsules that are now part of the UK national immunisation plan. However, these vaccines confer no protection against strains with a serogroup B capsule, the most frequent cause of meningococcal disease in Europe and North America. The B capsule has the identical chemical composition as a human post-translational modification, so the bacterium deploys its capsule to mimic a host molecule to avoid detection by the immune system. This precludes the use of the serogroup B capsule as an antigen for vaccination, given the potential risk of eliciting autoimmunity.

We have investigated the mechanisms by which *N. meningitidis* evades the human complement system. Complement is an evolutionarily ancient component of innate and adaptive immunity, that leads either to lysis of microbes or their engulfment and destruction by phagocytic cells. The striking predilection to meningococcal disease of individuals with rare defects in the complement system highlights the importance of this aspect of the immune system in protection against *N. meningitidis*. Rachel Exley in the group demonstrated that the ability of the meningococcus to evade human complement is governed by its metabolism and its ability to recruit complement factor H (CFH) to its surface. CFH is an abundant protein in serum which interferes with complement activation on the vascular endothelium to prevent self-damage.

Human CFH is bound by the bacterium by a lipoprotein, factor H binding protein, fHbp (Figure 1). fHbp is a highly polymorphic protein, which consists of two beta barrels tethered to the surface of the bacterium by a lipid moiety which is covalently linked to the N-terminus of fHbp. We showed that fHbp binds specifically to human CFH at high affinity; indeed the bacterium has evolved to bind CFH at higher affinity compared with its natural ligands in the human body. Thus, the bacterium can scavenge CFH from its host and co-opt it for its own use. In this way, *N. meningitidis* cloaks itself in a human protein (reducing immune detection) while also switching off the complement system at the precise site where immune attack is initiated.

Independently, groups in Pfizer and Novartis (now GSK) identified fHbp as a vaccine antigen which is now included in two available vaccines against serogroup B *N. meningitidis.* The vaccine from GSK contains several antigens in addition to fHbp, as well as a preparation of outer membrane vesicles (OMVs), a complex mixture of antigens and bacterial endotoxin. The antigens are derived from a single strain of the meningococcus and were not selected based on the known diversity of the antigens. The Pfizer vaccine contains two versions of fHbp, which were chosen by examining the extent to which they confer cross protection against strains expressing different fHbps. Of note, fHbp from either vaccine is expected to bind to its human ligand CFH after injection which might affect immunogenicity.

We are developing fHbp-based vaccines in collaboration with the Serum Institute of India, the largest vaccine manufacturer globally.

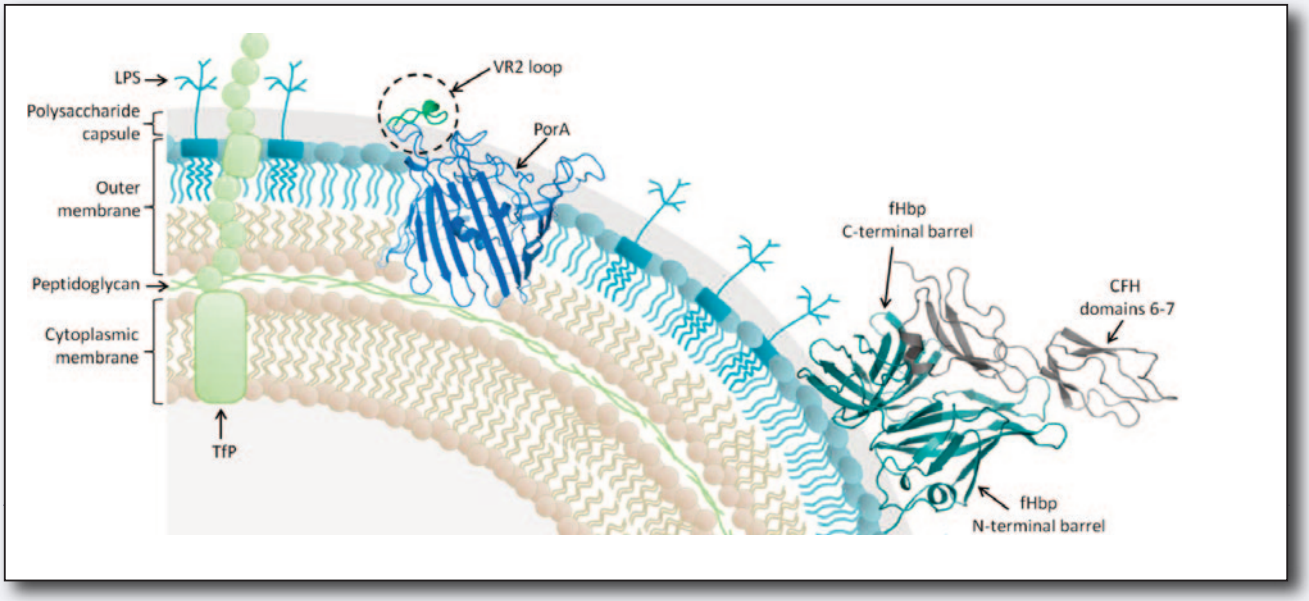


Figure 1. Architecture of the important meningococcal antigens fHbp and PorA, and their relationship with the bacterial surface. The VR2 loop is the immunogenic portion of PorA. LPS, lipopolysaccharide; Tfp, Type four pili; CFH, human complement factor H.

Instead of using wild-type fHbp, we have engineered fHbps which fail to bind CFH. We have identified the site on fHbp where CFH binds through structural and mutational analysis, and have generated non-functional fHbps, which have lost their activity while retaining their immunogenicity in a manner analogous to toxoids. To cover the degree of variation of fHbp and other antigens, we have exploited the Meningococcal Research Foundation Genome Library (MRF-GL) which I initiated in 2001 as Chair of the scientific board. The MRF-GL contains whole genome sequence data from every meningococcal strain that causes disease across the UK and harbours sequences from over 10,000 isolates. The library is housed by Martin Maiden's laboratory in the Department of Zoology and is a collaboration that includes his group, Public Health England, and the Sanger Centre. The MRF-GL provides a unique resource for understanding the meningococcal population structure and its relationship with sequence diversity of vaccine antigens. Therefore, we have been able to choose rationally fHbp variants for inclusion in a vaccine. Given that there are well over 1000 sequence variants of fHbp in the MRF-GL, judicious choice of fHbps (based on their relatedness and tests of immunogenicity) is essential so that immune responses will cover the diversity of *N. meningitidis* and thereby provide effective vaccine coverage.

However, there is an inherent concern in developing a single antigen vaccine against a rapidly evolving and diverse pathogen. This has been exemplified by the emergence of Covid-19 expressing spike protein variants in the past year which threaten to undermine first generation vaccines. Of particular concern for fHbp, we identified strains of the meningococcus that do not express fHbp yet still cause disease. We have, therefore, included protective epitopes from a second antigen, PorA, in our vaccines along with fHbp. PorA is a hydrophobic protein which is largely embedded in the lipid-rich environment of the outer membrane (Figure 1) and is, therefore, difficult to make and purify at high levels in the lab. Instead of using the whole protein, we have selected the surface exposed loops of PorA (consisting of 8-22 amino acids) and grafted them into sites in fHbp so they are presented to the immune system in their native conformation. In this way, we have used our knowledge of the structure of these antigens to generate chimeric vaccine antigens, using fHbp as a molecular scaffold to display immunogenic epitopes of PorA. These single protein antigens elicit immune responses against two separate antigens on the bacterial surface and our choice of fHbp and PorA has been informed by the database of WGS information at the MRF-GL. We are working on the final formulation of antigens with the Serum Institute of India before we embark on clinical trials.

N. gonorrhoeae causes the sexually transmitted infection, gonorrhoea, and has developed resistance against all classes of antibiotics. There was a widely held view that it would be impossible to develop vaccines against the gonococcus. This stemmed from the lack of protective immunity following natural gonococcal infection, and a series of failed clinical trials with prototype vaccines. However, there has been a recent resurgence of interest in gonococcal vaccines. Research has uncovered the multiple mechanisms deployed by the gonococcus to escape immune surveillance by surviving within neutrophils and by subverting T cell responses. There is also tantalising, but as yet unconfirmed, evidence that meningococcal outer membrane vesicles (OMVs) (Figure 2) can protect against gonococcal disease. The national roll out of an OMV-based meningococcal vaccine in New Zealand was associated with a small reduction in the rate of gonorrhoea among the vaccinees. Therefore, my group decided to initiate studies of gonococcal population biology with Martin Maiden and Eduard Sanders (who is based in Kilifi Kenya), and developed tools for studying the bacterium in the laboratory led by Ana Cehovin.

Our preliminary work led to the Gonococcal Vaccine Initiative (GVI), a collaborative project funded by the Wellcome Trust. The GVI will run over four years in the first instance, and includes scientists from Manchester (Professor Jeremy Derrick, a structural biologist), the USA (Professor Ann Jerse, an expert in gonococcal infection biology) and Kenya (Eunice Nduati and Eduard Sanders). Our overall aim is to design and evaluate vaccine candidates based on population genetics, structural biology, immunology and results from a clinical study. The latter will analyse the immune responses to meningococcal antigens

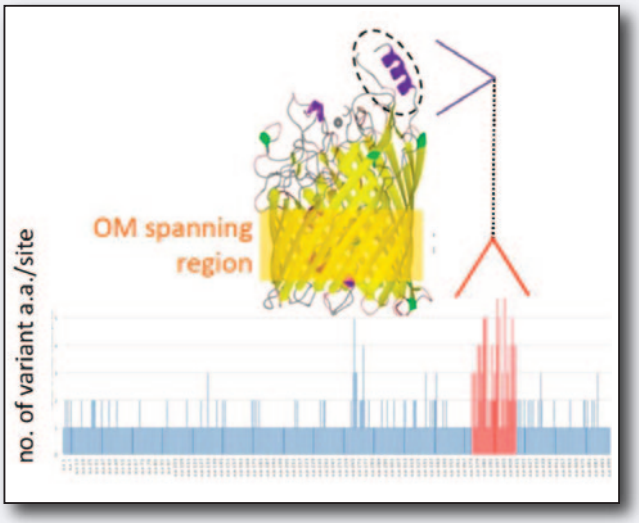


Figure 2. Variation in the sequence of a gonococcal surface protein shown by the number of variants in over 3,000 isolates found in the PubMLST database, with the most variable region (in red) mapped onto the structure of the antigen. The region occupied by the outer membrane is shown.

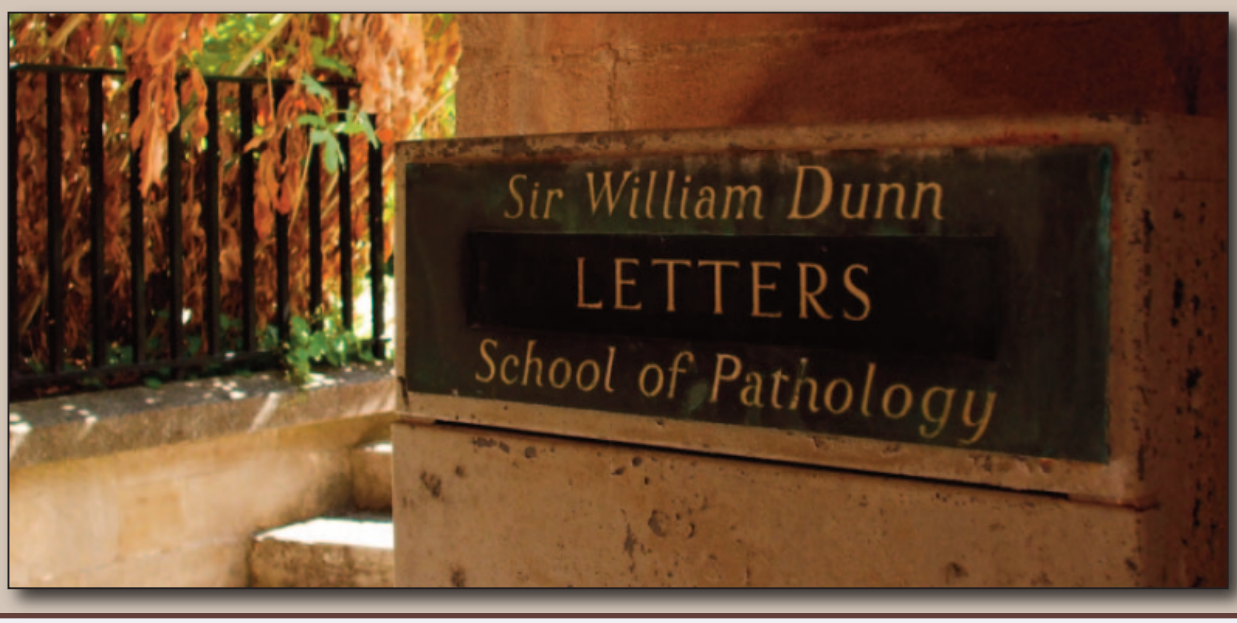
among sex workers in coastal Kenya; this group has been previously exposed to the gonococcus and is, therefore, more likely to generate immune responses. The clinical trial should provide unique insights into the antigens recognised by an at-risk population. Although this project is at an early stage, we have promising leads which can confer a degree of protective immunity.

Finally, what are the main priorities for vaccine development in the area of infectious diseases, and what advances are we likely to witness over the coming decade? Clearly AMR pathogens are a category of bacteria of increasing concern. It is estimated that our inability to treat AMR pathogens leads to over 700,000 deaths annually, a number that is likely to be an underestimate and to increase; much of the burden of AMR bacteria falls in countries where there are inadequate diagnostic facilities and poor recording. Resistant organisms such as *Klebsiella*, *Pseudomonas*, and *Escherichia coli* pose a particular threat to the elderly and immunocompromised individuals, who are less able to mount effective immune responses; for these pathogens, it will be a challenge to induce protective immunity in at risk populations. Another area emerging as a major focus of attention is that of chronic infections. An effective subunit vaccine against Herpes zoster (which causes chicken pox and shingles) and promising data for tuberculosis and malaria have arisen through the use of modern adjuvants, which activate the innate immune system so that adaptive immunity is triggered following exposure to antigen(s) in the vaccine. Combined with the wealth of information from genome sequences and our increasingly sophisticated knowledge of host-pathogen interactions, there are likely to be many exciting breakthroughs in vaccinology over the next 10 years.

Join the Dunn School Alumni Network

In the build up to our centenary celebrations in 2027, the Dunn School is keen to reconnect with our old members. We have established a new mailing list through which we hope to let our alumni know of news, events and preparations for the centenary. The mailing list will be complementary, but not overlapping, to the annual edition of Fusion, and will also allow us to contact you with more time-sensitive information.

The Dunn School Alumni Network is open to all former staff and students, in any capacity or role in the department. If you would like to sign up to receive our email updates, please visit: www.path.ox.ac.uk/content/alumni-network.
If you have any queries or suggestions regarding the Dunn School Alumni Network or our upcoming centenary celebrations, please contact alumni@path.ox.ac.uk.



FOCUS ON DNA

KATalysing Genome Protection: When Acetylation Fine-tunes Chromatin Association

Marjorie Fournier and Fumiko Esashi

Our DNA gets damaged…

DNA constitutes the genetic material which is important for the survival and functioning of each cell in our bodies. DNA damage therefore risks causing genetic changes that can lead to the development of diseases such as cancer. It is estimated that, in human cells, tens of thousands of DNA lesions occur daily[1], which represents a huge challenge for the ongoing functioning of the body. DNA damage can be induced during normal cell division and metabolic activities, but also in response to environmental factors, such as radiation or genotoxic agents. The extent of DNA damage is diverse, from less harmful oxidation of DNA bases caused by exposure to reactive oxidative species, to DNA double strand breaks, the most deleterious DNA damage, induced, for example, by ionising radiation.

…but fortunately, cells can repair our DNA

To face the challenges associated with DNA damage, human cells have evolved various repair mechanisms depending on the type of DNA lesions. For example, base excision repair can remove chemically modified or oxidised bases. Nucleotide excision repair allows for the removal of UV-damaged DNA. Cells are also equipped with a sophisticated error-free mechanism to repair DNA double strand breaks (DSBs), called homologous recombination (HR). During HR repair, the broken DNA is first resected to generate single stranded DNA (ssDNA), which then 'search' for a region of sequence homology, normally within the replicated sister chromatid. This is followed by the process of 'copying' intact genetic information from the sister intact chromatid DNA, which is then 'pasted' back to the region of broken DNA. In this way, HR enables the repair of DSBs without errors[2].

The initial process of HR repair is essentially catalysed by an evolutionarily conserved recombinase, called RAD51, assisted by the breast cancer susceptibility proteins BRCA2 and its binding partner PALB2 (partner and localiser of BRCA2). Hereditary mutations of *BRCA2* and *PALB2* genes increase the risk of developing various cancers, most notably breast, ovarian and pancreatic cancers[3-5], and Fanconi anaemia, a rare genetic disorder characterised by bone marrow failure, developmental abnormalities and high incidence of childhood cancers[6]. These observations demonstrate the importance of BRCA2 and PALB2 in the prevention of human diseases. Importantly, there is strong evidence that *BRCA2*- and *PALB2*-defective cells show increased sensitivity to a specific type of drugs, blocking an action of poly (ADP-ribose) polymerase 1[7], an enzyme which facilitates non-HR DNA repair mechanisms. Such drugs have been approved for the therapeutic treatment for patients who have defects in HR-associated genes. In the past decade, however, it has become increasingly clear that the impact of PARP1 inhibitors is transient and HR-defective cancer cells often develop drug resistance over the time.

Our laboratory has been working to understand how BRCA2 and PALB2 act to maintain the genome in human cells. We study their functions in HR repair and beyond to fully understand syndromes associated with *BRCA2*- or *PALB2*-defects, which cannot be explained simply by defects in HR repair. In doing so, we hope to enhance our understanding of their functions in global genome protection, and then to assist the development of therapies against these syndromes associated with defects in these proteins.

PALB2 safeguards our genome for constant protection

A group of chromatin-associated proteins provides constant genome surveillance even in the absence of external genotoxic agents, such that it allows for DNA repair as soon as damage occurs. PALB2 is one of these factors and associates with chromatin via two distinctive mechanisms; 1) direct association with nucleosomes though the chromatin-association motif (ChAM), a conserved domain uniquely found in PALB2 across species[8], and 2) association with MRG15, a chromodomain containing protein which recognises a marker of active transcription, namely tri-methylation of histone H3 at the position of lysine 36 (H3K36me3). Transcription can pose threats to DNA even in normally growing conditions, particularly when DNA replication takes place within these regions. We found previously that the constitutive presence of PALB2, tethered by MRG15, monitors the status of actively transcribed regions and prevents DNA damage that can otherwise be induced due to the transcription-replication collision[9]. Intriguingly, upon the induction of DSBs, for example by exposure to ionising radiation, PALB2 is released from these actively-transcribed regions and newly recruited to exact sites of DNA damage. PALB2 protein is not abundant in cells, and this mechanism appears to assist RAD51 to initiate HR repair upon DNA damage. These two modes of PALB2 chromatin association – one at transcriptionally-active sites, and another at sites of DSBs – are both important for genome maintenance, but we still do not understand the mechanism by which the modes of PALB2 chromatin association are switched from one to the other, and how PALB2 becomes mobilised as and when required.

Lysine acetylation as key molecular switch of PALB2 chromatin association

Post-translational modifications (PTM) play essential roles in cell signalling and in regulating protein function and these are especially important during the DNA damage response[10]. Interestingly, by analysing all acetylated proteins in normally growing human cells, we discovered that the ChAM domain of PALB2 was targeted by essential

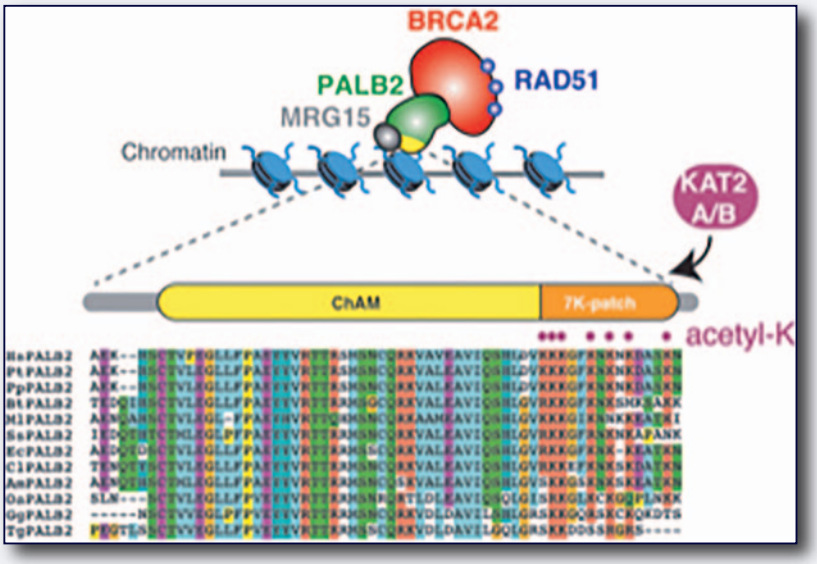


Figure 1. Depiction of the seven lysines residues (7K-patch) acetylated by KAT2A and KAT2B within ChAM domain of PALB2 in complex with MRG15, BRCA2 and RAD51 at transcribed chromatin. Acetylated lysine residues are highlighted by purple circles. ChAM protein sequences from twelve PALB2 orthologues Hs *(Homo sapiens, human),* Pt (*Pan troglodytes,* chimpanzee), Pp (*Pan paniscus,* bonobo), Bt (*Bos taurus,* cow), Ml (*Myotis lucifugus,* little brown bat), Ss (*Sus scrofa,* wild boar), Ec (*Equus caballus,* horse), Cl (*Canis lupus familiaris,* dog), Am (*Ailuropoda melanoleuca,* giant panda), Oa (*Ovis aries,* sheep), Gg (*Gallus gallus,* red junglefowl), Tg (*Taeniopygia guttata,* zebra finch) are shown.

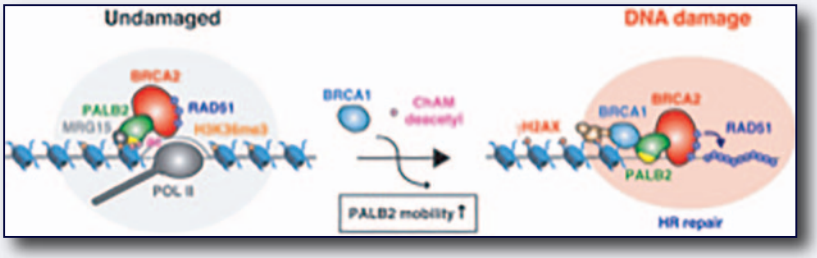


Figure 2. Model for PALB2 acetylation function in genome protection. MRG15 and KAT2A/B-mediated ChAM acetylation jointly promote PALB2 enrichment at undamaged transcriptionally-active chromatin. DNA damage triggers ChAM deacetylation and releases PALB2 from chromatin. This allows PALB2 to interact with BRCA1, which in turn recruits the entire HR complex to sites of DNA damage. ChAM binding to naked DNA through the 7K-patch may promote RAD51 loading and HR repair.

PTM enzymes, KAT2A and KAT2B, which introduce an acetyl-group to lysine residues in proteins[11]. These enzymes, also known as GCN5 and PCAF, respectively, are well characterised for their roles in regulating transcription and maintaining genome stability[12]. More recently, we found that the PALB2 acetylation is particularly evident at a cluster of seven lysines (7K-patch) within the ChAM, and that ChAM acetylation promotes PALB2 chromatin association (Figure 1)[13]. DNA damage, however, triggers the rapid removal of acetylation from ChAM, the increase in PALB2 mobility and the dissociation of PALB2 from chromatin. Indeed, the substitution of the lysine residues within the 7K-patch with alanine residues that cannot be acetylated, impaired PALB2 chromatin association. Conversely, the 7K substitution to another type of residue, glutamine, which is often considered to mimic acetylated lysine, rendered PALB2 functional in HR repair, leading to an increase in cellular sensitivity to PARP inhibition.

Based on these observations, we propose the following model: in undamaged cells, acetylation of PALB2 within the 7K-patch assists its constitutive association with chromatin. PALB2 is retained at actively transcribed regions, likely enforced by the local activities of KAT2A and KAT2B at these regions, to reduce the risk of DNA damage due to the collision between transcription and replication machineries. Conversely, DNA damage, induced by external sources, triggers the removal of the 7K-patch acetylation, which then promotes its dissociation from undamaged regions of chromatin. This increases PALB2 mobility, such that it can be efficiently recruited to DNA damage sites to promote HR repair (Figure 2). Overall, both switching on and off the lysine acetylation at the ChAM domain appear important to modulate PALB2 affinity with chromatin, hence defining the mechanism of genome protection by PALB2 at either undamaged or damaged chromatin.

Considering the clinical relevance of PALB2 and the importance of lysine acetylation in regulating its function in genome protection, the next important step would be to investigate the link between the levels of acetylated PALB2 and cancer development. While current cancer therapies targeting the HR pathway using PARP inhibitors often suffer from the emergence of resistance, combined therapies with those targeting acetylation signalling pathways may prove effective in treating a certain group of patients. Indeed, small molecule inhibitors targeting lysine deacetylases or bromodomain inhibitors are already in clinical trials. It would also be important to consider targeting metabolic pathways that generate the acetyl-CoA required for acetylation, which can be modulated simply by diet.

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FOCUS ON DNA

DNA Replication, Singled Out

Carolin Anne Müller

By the time you finish reading this sentence, your body will have produced 5 million new cells. In adults, nearly two trillion cells divide every day. Prior to each cell division, it is essential that all the genetic material is copied exactly once.

Making one copy sounds manageable. But making one *exact* copy of 6.4 billion base pairs in 8 hours (i.e. 10 million per minute), without *any* mistakes sounds like wizardry! Decades of research have unravelled some of the mystery, but many aspects remain elusive.

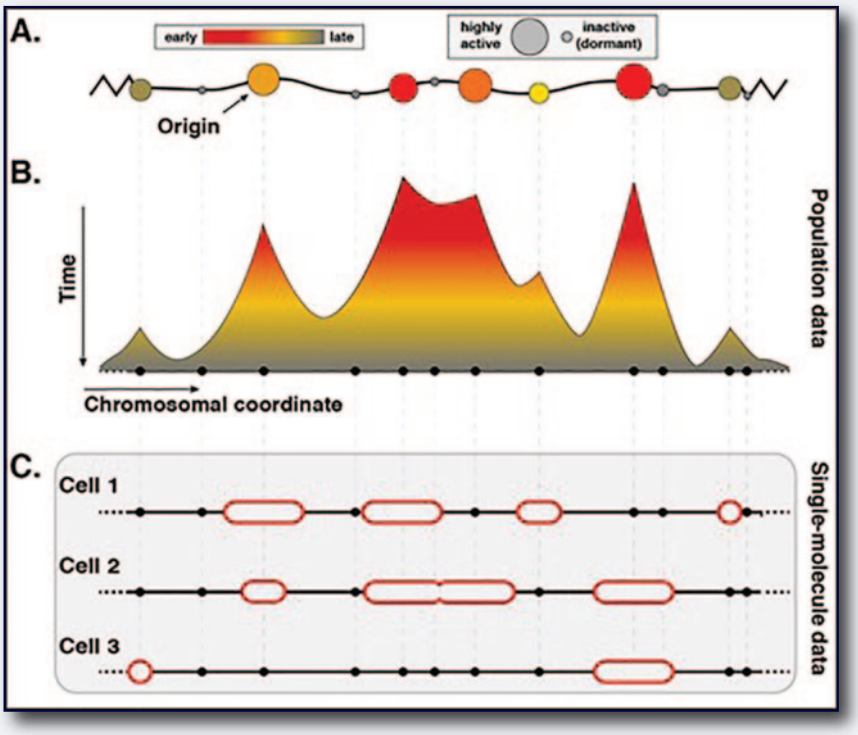

Figure 1. Eukaryotic genome replication. (A) A cartoon showing replication origins at multiple chromosomal locations. (B) Eukaryotic chromosomes replicate in a defined, reproducible temporal order, dictated by the location and activity of replication origins. The time of replication can be measured genome-wide using millions of cells. (C) Population ensemble data hide the heterogeneity between individual DNA molecules.

Understanding DNA replication was off to a good start with Watson and Crick's observation, "*It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material*"[1]. Only a few years later, Meselson and Stahl confirmed the semi-conservative nature of DNA replication[2]. Nowadays, every school child is taught the basic principles – the DNA double strand is unwound and replicative polymerases synthesise nascent DNA using the parental strands as templates. The initial unwinding occurs at so-called DNA replication origins, which are specialised DNA sequences that are recognised by replication proteins. Upon activation of these proteins, two replication forks are assembled and begin translocating in opposite directions away from the origin, replicating the intervening DNA. Recently, several laboratories succeeded in reconstituting DNA replication initiation, elongation and termination *in vitro*[3-5], thus adding molecular detail to the canonical textbook image. We can now name the essential proteins, delineate their interactions and describe their regulatory mechanisms. However, having the essential parts and a manual are not sufficient to solve one of nature's greatest mysteries – how do cells achieve complete genome replication?

Like all great magic tricks, timing is critical. Genome replication is governed by two time constraints. Firstly, it can only occur during S phase of the cell cycle. In this defined window of time, cells have to ensure complete, and precise genome duplication. This is achieved through the usage of many replication origins that are distributed along every eukaryotic chromosome (Figure 1A), ranging from hundreds in yeast to thousands in human cells. However, only a subset of the available replication origins are used per cell cycle. The remaining origins can act as a backup mechanism in case replication forks stop prematurely. Replication more than once or, conversely, failure to complete replication before mitosis, can lead to genome instability and genetic diseases, and thus must be avoided at (almost) all cost.

The second time-related constraint is on the number of replication forks that can be active simultaneously. A moving fork requires nucleotides, the building blocks of DNA, to synthesise the nascent strand. Exhausting the supply of nucleotides would slow down polymerases and leave the unwound DNA exposed to potential damage. The only way to limit the number of ongoing forks is by regulating origin activation. Once a certain number of origins has initiated replication, the other origins in that cell are blocked from activating until some forks have terminated. This cascade of origin activation leads to differences in the replication time across the genome, with regions close to early origins being replicated first, and regions far from origins replicating last (Figure 1B).

So, is replication time indeed critical? In other words, are differences in replication time physiologically relevant, or are they merely a solution to the supply-and-demand problem? To answer this question (and many more), our group has developed methods to accurately measure the replication time genome-wide, using high-throughput sequencing

technologies[6]. We applied these methods to the model organism *Saccharomyces cerevisiae* and discovered that genome replication dynamics are very reproducible, evolutionarily conserved, and remarkably resilient to most genetic manipulations and environmental changes[7,8]. Many of our observations have since been confirmed by groups working with other organisms, including mammalian cells. Yet, one key feature remains unique to *S. cerevisiae* – our ability to remove replication origins and thus delay the replication time at specific loci of interest, with only a few targeted point mutations. That way, we forced centromeres, which are among the earliest replicating sequences in the genome, to replicate late and *Eureka!* genome instability[9]! We also revealed a direct link between gene replication timing and gene expression when we delayed histone gene replication[10]. In conclusion, regulated replication timing is indeed important for genome stability.

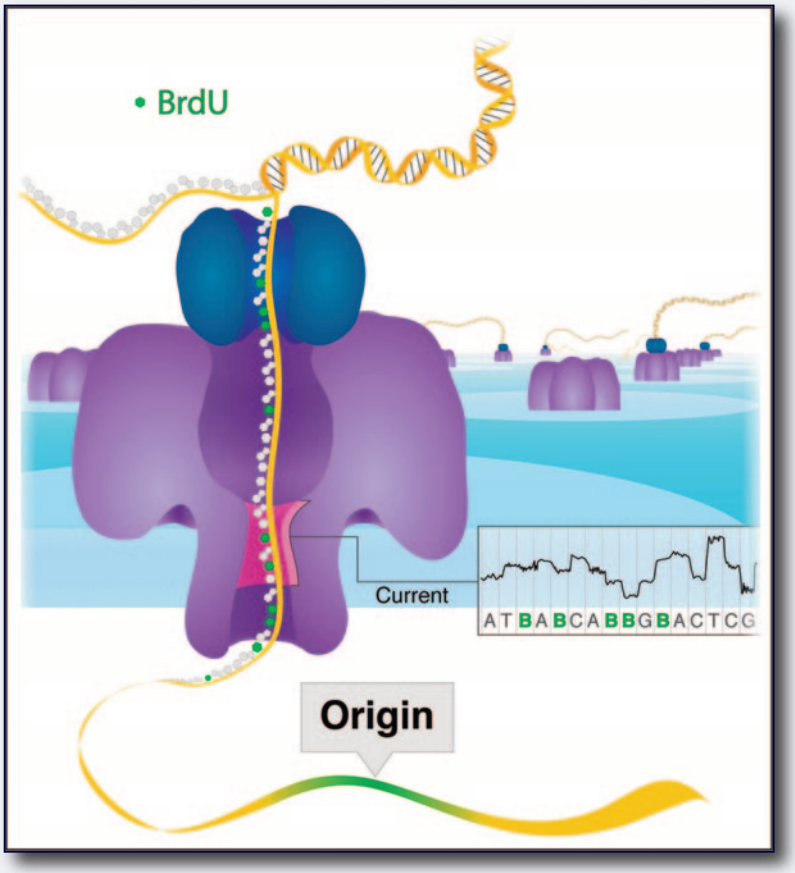

Figure 2. DNA sequencing using a MinION. A processive enzyme (dark blue) ratchets DNA into the pore (purple), with the base sequence in the central channel (pink box) determining the output electrical current. The electrical current signature of BrdU can be distinguished from thymidine, allowing identification of regions replicated during a BrdU-pulse.

As mentioned above, genome replication timing measurements are surprisingly reproducible and robust, implying a significant degree of homogeneity between cells. In fact, the opposite is true. No two cells will replicate their genomes with exactly the same kinetics, because the subset of origins that is used and their activation times differ in every cell cycle (Figure 1C). This discrepancy between experimental observation and reality is due to technical limitations. To measure replication timing genome-wide, we have to pool millions of cells to get sufficient material. Thus, the data is a population average, which hides the cell-to-cell variability, including rare pathogenic events. However, until very recently, there were no methods to study genome replication in single cells or molecules. The options were to analyse a single, previously designated genomic location one cell at a time, or to microscopically image individual DNA molecules of unknown genomic location. Our lab decided to address this shortcoming and develop a high-throughput, single-molecule method to measure genome replication dynamics.

Our method uses the 'MinION' sequencer recently developed by Oxford Nanopore Technologies. The MinION determines the DNA sequence by measuring base-specific changes to the electric current as the DNA molecule passes through a nanopore channel (Figure 2). The sequenced molecules can be extremely long (>1 megabases) and they do not require amplification prior to sequencing. Thus, *in vivo* modified bases, including methylated or damaged bases, can be sequenced and detected directly. Base modifications in the form of base analogues are commonly used as tools to study DNA replication. The analogues, for example the thymidine analogue BrdU, are taken up by cells and incorporated during genome replication, thus 'labelling' newly synthesised DNA. Our MinION sequencing approach directly detects BrdU at base-resolution on single molecules.

Once again, we turned to *S. cerevisiae* and its wealth of available resources to establish our high-throughput genomics approach to study DNA replication on single molecules, called D-Nascent11. Exposing cells to a limited concentration of BrdU at the very start of S phase revealed genome-wide origin firing and replication fork dynamics on molecules up to 300 kb in length. The single molecule nature of our approach uncovers the cellular heterogeneity in genome replication dynamics and enabled the discovery of a new class of replication origins in budding yeast. This discovery redefines our understanding of genome replication in *S. cerevisiae* – the eukaryote in which genome replication is best characterised. One can only speculate what secrets D-NAscent might unlock once we achieve our current goal of adapting it to human cells. Maybe one day, we can solve the mystery of genome replication, but that will not make it any less amazing.

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HISTORICAL PERSPECTIVE

In the first of a new series, *Fusion* approached senior members of the Dunn School to ask them to describe the research for which they are best known. Here Herman Waldmann describes his journey to uncover the mechanisms of immunological tolerance.

Tolerance Can Be Infectious

Herman Waldmann

It is now some 8 years since I had the pleasure of writing the editorials for *Fusion* summarising all the wonderful activities being undertaken at the Dunn School. Now, deep into my old age, Paul Fairchild has asked me to write what memories I still retain about the discoveries I am most proud of. "Proud" is a peculiar word, implicating some self-congratulation. So please do forgive me if, instead, I write about the scientific theme that has directed our research through uncharted territories over the years, providing both intense intellectual satisfaction as well as the opportunity to interact with many fine colleagues, in ways I could never have anticipated.

As a pre-clinical medical student, I had my first stringent taste of research in the Cambridge Natural Sciences Part II course. There I learned that many hours of hard endeavour did not guarantee a breakthrough discovery, and that I should have given more of the time to theory and planning. Yet in a perverse, somewhat masochistic way, I had enjoyed that challenge.

As I continued to pursue my clinical studies at a London hospital, I become aware of how inadequate the medicines available at that time were for controlling unwanted inflammation. I sensed that a deeper knowledge of the immune system would enable the discovery of improved treatments. I therefore decided, immediately after qualifying clinically in 1971, to return to Cambridge to gain PhD training in immunology.

At that time, following the discovery of clonal selection as the basis of how lymphocytes could respond to so many diverse antigens, it seemed that understanding the mechanisms underlying lymphocytes' interactions with each other, might provide a good starting point for 'translational' opportunities. An important feature of clonal selection was that all lymphocytes were pre-committed to recognise just one antigen as a result of having unique antibody-like receptors. They used the same receptor to both switch on their response to antigen, and, in other circumstances, to switch it off, a seemingly necessary requirement to avoid autoimmune diseases.

The fashionable questions around at that time related to how the immune system could cope with so many different microbial threats, without damaging *self*. In experiments using newborn mice, Medawar had already shown that self-tolerance was acquired rather than genetically inherited, through exposure to antigens early in lymphocyte development. Consequently, the popular hypotheses for how individual lymphocytes decided whether to respond to antigen or abort, focussed around their state of maturation, and/or the need for further triggering signals. It seemed likely that understanding how lymphocytes made that decision would have enormous therapeutic implications in enabling acceptance of foreign tissue transplants and reversal of autoimmune diseases. The predominant candidate for a source of those additional signals was the so-called *helper* T cell, known to collaborate and supply "helpful" stimuli to both B cells and other T cells. Insufficient *help*, it was thought, predisposed lymphocytes engaging antigen to tolerance. My PhD goals were to define the molecular basis of that *help*.

While I was finding my feet in both theory and laboratory methods, I was very fortunate that Alan Munro, my PhD mentor, had arranged for me to meet an American immunologist, Jacques Chiller. Chiller and his collaborator Weigle, had just reported that adult mice could be rendered immunologically tolerant of a foreign protein, if that protein were freed of all aggregates. Their work had shown that lymphocytes, even in an adult animal, could be tolerised by antigen.

Two guiding principles then became clear to me. First, that robust therapeutic interference with lymphocyte cooperation might enable tolerance induction in a mature immune system. Second, any therapeutic strategy for the induction of tolerance for therapeutic purposes would need to take account of the sustained output of new T cells from the thymus, way after therapy had stopped. In other words, any therapeutic strategy had to establish and enforce a long-term program for maintaining tolerance once induced.

Around this time, two immunologists, Wolf Droege and Richard Gershon, published papers suggesting the existence of *suppressor* T cells that could inhibit immune responses. Were these cells contributing to self-tolerance? Their publications became the basis for much controversy fuelled by the lack of reagents to separate the postulated *suppressor* T cells from helpers. Gershon claimed that the *suppressor* T cells expressed the membrane CD8 molecule, hitherto thought of as marker for cytotoxic T cells. However, this proved difficult to substantiate in other experimental systems.

The discovery and development of monoclonal antibodies (mAbs) by Cesar Milstein rejuvenated immunology and offered beautifully precise reagents to 'dissect' complex systems such as those involved in immunological tolerance. Not only did they provide tools to track, purify or ablate cells with defined functions, but also offered the potential for "magic bullet" precision therapeutics.

With long-term MRC support, our team generated mAbs to mouse and human T cells so as to dissect out functionally distinct T cell populations. Amongst these new mAbs were some defining mouse *helper* T cells (anti-CD4 mAbs) and others targeting mouse cytotoxic T cells (anti-CD8 mAbs). Using these, we could test the hypothesis that tolerance might be inducible in adult mice by a lack of T-cell *help*. In

1986 we published the first of a series of papers showing that anti-CD4 therapy would enable tolerance to a foreign protein that Chiller had used as his immunogen. Soon after, we showed that a combination of CD4 and CD8 antibodies would bring about antigen-specific immunological tolerance to foreign skin transplants (Figure 1).

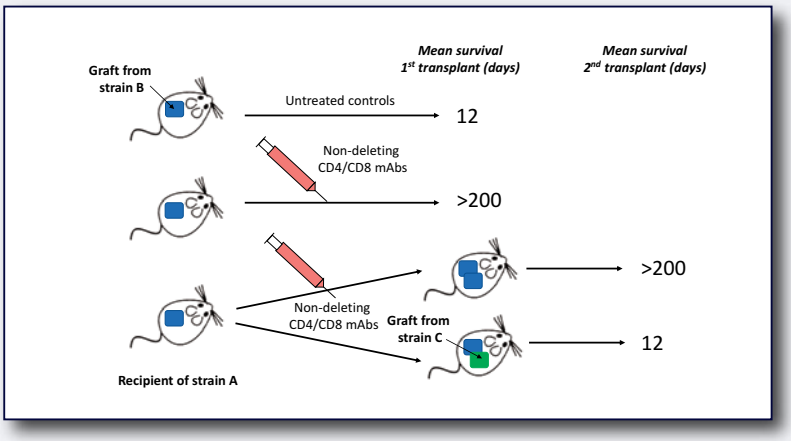


Figure 1. A cocktail of non-deleting monoclonal antibodies to CD4 and CD8 secure long-term survival of skin grafts from genetically dissimilar strains of mice. Once tolerant, mice may receive additional grafts from the same donor but reject grafts from third party.

In both cases, the induction of tolerance did not require destruction of the T cells, but merely blockade of CD4 and CD8 function. We rationalised that we were interfering with *help*, whilst holding back CD8 T cells from engaging in rejection until tolerance was complete. Consequently, it seemed as if the cooperation-based hypothesis was a front runner. One experimental finding was, however, not compatible with a simple idea of antigen-specific clonal abortion/deletion in the absence of *help*. We were finding that we could not break the induced tolerant state with infusions of fresh naive T cells. This phenomenon, which we coined *resistance*, needed to be explained.

This anomaly was dissected by making use of transgenic mice expressing a human protein (CD2) on all their T cells (shown as yellow in Figure 2). We tolerised the mice to a foreign skin graft and then, many weeks later, attempted to break that tolerance by infusing unmarked recipient-type T cells (depicted as blue cells). Tolerance remained intact! If, however, we first ablated the 'marked' recipient T cells from the tolerised mice (using an anti-human CD2 antibody), then the same unmarked T cell infusions rejected the previously-tolerated grafts. In the same type of experiment, we allowed the infused T cells to coexist with tolerised host T cells and the tolerated graft for some weeks before ablating host T cells. What we then found was that these T cells were now unable to reject the graft, and that they had become tolerant and suppressive in their own right (Figure 2).

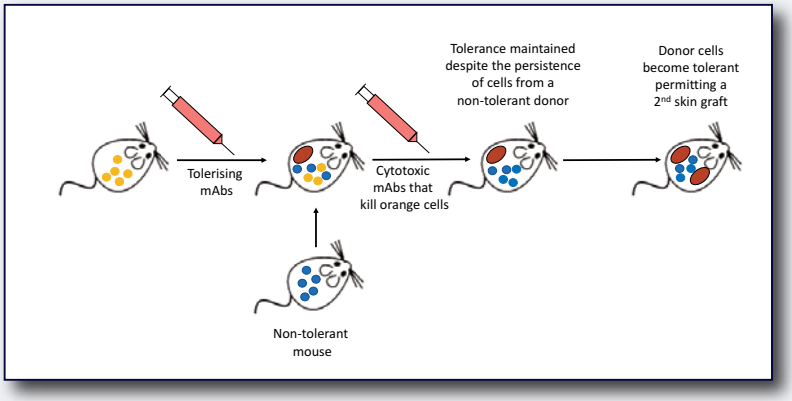


Figure 2. 'Infectious' tolerance. Once established through the administration of a cocktail of tolerising antibodies, tolerant T cells educate cells from a non-tolerant mouse to acquire a tolerant state.

Further experiments showed that the T cells that were suppressive as a result of antibody therapy, were $CD4^+$ and *not* $CD8^+$ as had been reported by Gershon many years earlier. We, perhaps unwisely, used the term *infectious tolerance* to describe its infectious nature, whereas Gershon had used that term simply to describe his experiments of T cell-mediated *suppression*. We paid a price for that in forfeiting some credit for the novelty of our findings.

Further work on the mechanisms of suppression revealed two key findings. The first was that regulatory T cells induced to one set of graft antigens could prevent rejection of grafts carrying additional 'third-party' specificities (a phenomenon we referred to as *linked suppression*). The second was the demonstration that tolerated grafts carry within them functional regulatory T cells. These two observations led us to conclude that regulatory T cells could operate within tolerated tissues by altering the tissue microenvironments to be non-permissive for rejection, a form of so-called *immune privilege*.

Notwithstanding the huge effort we had made producing mAbs specific for proteins on the T cell surface, we constantly failed to find any that were unique to cells that were suppressive in our transplant models. This changed when clinical and fundamental studies in other laboratories discovered that a transcription factor (Foxp3) endowed T cells with regulatory function, and indeed defined them. Together with Shohei Hori in Japan, we constructed a transgenic animal expressing the human CD2 protein under the influence of the FoxP3 promoter. This gave us what we needed to be able to study the biology of regulatory T cells: we could now track, purify and ablate them as needed with anti-human CD2 mAbs. Inevitably, in the modern era, we could now analyse the transcriptome, epigenome, proteome, lipidome, metabolome, and from these evolve novel ideas on how $FoxP3^+$ regulatory T cells performed their suppressive role.

Although the models we chose were in the field of transplantation, the principles of 'infectious tolerance' are proving relevant to other arenas of regulation in autoimmunity, allergy, cancer immunology and chronic infectious disease. The ability to 'tip' the host's immune response toward regulation has elicited new waves of research activity in generating novel therapeutic strategies to both suppress and enhance immune responses in more precise ways than hitherto.

I still recall that lunch with Jacques Chiller - on his part, a generous act to give precious hours to a student he had never previously met. Jacques, and the many immunologists of that era, had to conceptualize ideas on

tolerance with very little experimental data at hand; they had no mAbs or genetically-manipulated mice. From all the experimental work by many in the field, one can now say that self-tolerance depends on the stage of development reached by lymphocytes experiencing antigen; the lack of adjuvant additional signals, as had been predicted, but now, in addition, a sophisticated process of infectious tolerance, ensuring a long-term ceasefire in potentially vulnerable tissues.

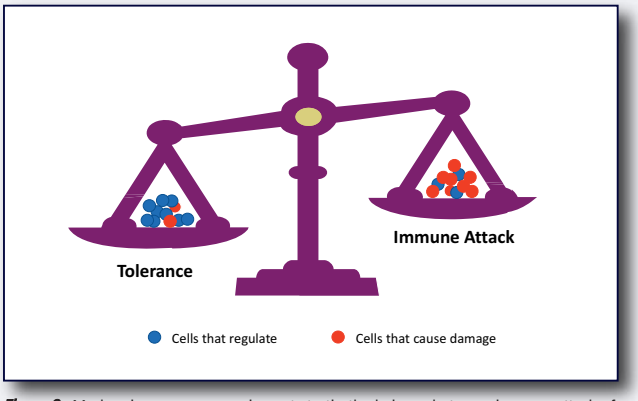


Figure 3. Modern immune suppression acts to tip the balance between immune attack of a transplanted tissue and long-term acceptance through immunological tolerance.

For any who wish to know more about the studies that clearly defined the existence of infectious tolerance, I would suggest the following references:

- Qin S, Cobbold SP, Pope H, Elliott J, Kioussis D, Davies J, Waldmann H (1993) "Infectious" transplantation tolerance. *Science* **259**:974-977.
- Kendal AR, Chen Y, Regateiro FS, Ma J, Adams E, Cobbold SP, Hori S, Waldmann H (2011) Sustained suppression by Foxp3+ regulatory T cells is vital for infectious transplantation tolerance. *J Exp Med* **208**:2043-2053.

For a listing of all the superb colleagues who accomplished these investigations over the years, and for more details on mechanisms, I would ask you to refer to:

- Waldmann H, Howie D, Cobbold S (2017) Induction of Immunological Tolerance. In: Gordon S (Ed) *Myeloid cells in Health and Disease.* Chapter 44. Wiley. ISBN:9781555819187 | doi:10.1128/9781555819194.

HISTORICAL PERSPECTIVE

John Radcliffe: His Life and Benefactions

Eric Sidebottom

John Radcliffe is certainly Oxford's favourite son. Many buildings are named after him and his legacies ultimately benefitted most people in Oxford. It is worth noting that the image of the Radcliffe Camera in Radcliffe Square is probably the most frequently used image of Oxford: indeed, I used it on the cover of my book *Oxford Medicine: A Walk Through Nine Centuries.*

John Radcliffe is one of the best-known names in Oxford and it is interesting to speculate why. He was enormously successful as a doctor both in Oxford and London, but didn't really discover anything significant. He didn't do important research but succeeded, instead, by observing others and copying what he thought good and important and avoiding what he considered bad, harmful or irrelevant procedures, such as bleeding and purging. He was socially adept, a good conversationalist and a generous host. There seems to be some uncertainty about the date of John Radcliffe's birth, either December 1652 or January 1653. It is recorded that he was baptised on 23rd January 1653. He was born in Wakefield where his father, George, a lawyer, was governor of the local prison.

John Radcliffe was fortunate to attend the excellent local schools (Queen Elizabeth Grammar School and perhaps Northallerton Grammar School) and so he was well prepared to move to University College, Oxford in 1665 at the early age of 12 or 13, where he was awarded a Freestones Foundation Exhibition (A Yorkshire charity). Obadiah Walker, senior fellow at Univ when he arrived and master from 1676, was a big influence on the young John Radcliffe and Radcliffe became a great support for Walker in later life. Indeed, when Walker died in 1698 he was living in Radcliffe's house in Carshalton.

John Radcliffe graduated with a BA in 1669 and became a senior scholar at University College. Unfortunately, no fellowships were available at Univ at that time and he therefore moved to a (Yorkshire) fellowship at Lincoln College in 1670. Here, somewhat surprisingly, he taught philosophy and logic while starting on his study of medicine. He graduated with an MA in 1672 and it was about this time that a college colleague described him as 'an illiterate sot'. It is interesting that in his essay, Quinton comments that there is probably a grain of truth in both these judgements! He was certainly a heavy drinker with a relatively small library!

Radcliffe continued his medical studies at Lincoln and graduated BM (Bachelor of Medicine) in 1675, after he had been at Lincoln for five years. He continued his medical studies until 1682 when he graduated DM (Doctor of Medicine). About this time, he fell out with the College, especially the Rector. The issue was about his future at College. If he was to remain a Fellow he was required to take Holy Orders. He was unwilling to do this and so soon afterwards he left Oxford and moved to London (in 1684). Here he spent the rest of his life, finding favour with royalty and high society from whom he received generous payments for his (generally rather conservative) medical treatments. He clearly was not popular with some of London's leading doctors who generally took a more radical (and less successful) route to treatments of their patients. Radcliffe soon attracted the attention of Royalty including William III and

Anne. He is reported to have offended them both, the King by commenting to him that "I would not have your two legs for your three kingdoms", and Anne by refusing to see her when summoned.

He died (at home in Carshalton), probably from a stroke, on 1st November 1714 at the age of 62, leaving a fortune estimated to be about £140,000. His funeral was a rather grand affair held in St Mary's Church in Oxford on 3rd December 1714. In spite of the impressive ceremony, no memorial was erected and his grave was left unmarked. It was rediscovered by chance in 1819 but a memorial tablet was not installed until 1953.

It is probably fair to say that the University's hopes and expectations were more than satisfied by his legacies. The first to be revealed was the Travelling Medical Fellowship programme where Fellows were appointed for 10 years, at least half of which was to be spent abroad. This fellowship programme is still active today, but the fellowships are now for 3 years only. It has been said that London has benefitted more than Oxford from this program but Radcliffe would no doubt be delighted by its overall success.

The grandest of his benefactions was probably initiated in 1737 when the foundation stone of the Radcliffe Camera was laid and £40,000 assigned to the project. The building was completed in 1747 and opened with due ceremony in 1749. It is interesting to note that the freehold of this building did not pass into the University's hands until 1927 when it first became a part of the Bodleian library.

It is fascinating now, three centuries after he was in practice, to speculate on how good a doctor he actually was. It is clear to me that I have become more charitable in my judgement with passing years. It is clear that he observed the practice of his colleagues carefully and learned much from them, especially what *not* to emulate. At the time he was training in Oxford, there were many active scientists, such as Boyle and Hooke and doctors, such as Sydenham and Willis, many of whom soon moved to London and eventually formed the nucleus of the Royal Society.

The first statue of Radcliffe was erected in Radcliffe quadrangle in University College, probably in 1714, as the college was being built. The unveiling of the latest statue, situated at Green Templeton College, was on the 25th September 2018. This statue was commissioned as part of the commemoration of the Tercentenary of The Radcliffe Trust.

Figure 1. 'Blue plaque displayed on the former Radcliffe Infirmary commemorating the first clinical use of penicillin prepared at the Dunn School.

Figure 2. Martin Jennings' statue of John Radcliffe in front of the Observatory at Green Templeton College.

The Trust has a long history with the city and University of Oxford having provided funding to build the Radcliffe Camera, the Radcliffe Observatory and the Radcliffe Infirmary, where, famously, penicillin manufactured at the Dunn School was first administered to patients for the treatment of bacterial infection, a landmark event appropriately commemorated with a blue plaque (Figure 1). Given the Trust's Tercentenary, it seemed appropriate for this connection to be commemorated with a new statue in memory of Dr Radcliffe. To that end, The Radcliffe Trust commissioned Martin Jennings to produce a suitable image of Radcliffe (Figure 2). Martin Jennings is a well-known and celebrated sculptor, having created works of many famous people including Mary Seacole, situated outside St Thomas' Hospital; Charles Dickens in Portsmouth; and George Orwell outside Broadcasting House. On the occasion of its unveiling, Professor Denise Lievesley, the Principal of Green Templeton College reflected: "It is a great honour that the Ashmolean, through the generosity of The Radcliffe Trust, has commissioned this sculpture to stand in front of the Radcliffe Observatory, the focal point of our College. We are especially proud to give a home to this masterpiece by Martin Jennings which adds John Radcliffe to his works of consummate art sited in prominent public places to celebrate the lives of luminaries in our history." A bronze maquette of the statue is now on display in the Ashmolean Museum.

Sources

This short biography is largely based on an article by Anthony Quinton published in the *Journal of the Royal Society of Medicine* (1986) **79**:380-386. For further material, I have also relied on books by Ivor Guest (1991) and David Cranston (2013).

Women scientists in the Dunn School and beyond played critical roles in the early studies and development of penicillin as a medicine.

HISTORICAL PERSPECTIVE

As the department prepares to celebrate the centenary of the opening of the Dunn School building in 1927, Keith Gull reflects on two aspects of the penicillin story that played out within its walls: the invaluable role played by women in its development and the award of the Nobel Prize to Fleming, Florey and Chain.

The Role of Women in the Development of Penicillin

Keith Gull

The 'Penicillin Girls'

When the spectacular anti-infective properties of penicillin were established by the team led by Howard Florey and Ernst Chain at the Dunn School there was a need for a rapid expansion in the supply. That meant growing the *Penicillium* mould in increasing quantities and Norman Heatley was responsible for much of this innovation. In essence, this led to a "factory" approach that desperately needed more people. The MRC provided funds for two assistants who came from nursing backgrounds – Ruth Callow and Claire Inayat. They were so successful that Florey recruited four others, Betty Cooke, Peggy Gardner, Megan Lankester and Patricia McKegney. Their efforts increased the supply of penicillin by a thousand-fold and collectively they became known as the 'penicillin girls' (Figure 1).

Figure 1. *'Two of the penicillin girls culturing* Penicillium *in the famous bedpans from the Radcliffe Infirmary, first commandeered by Norman Heatley for the purpose.*

Ethel Florey

Ethel Florey

Howard Florey met his first wife, Mary Ethel Hayter Reed (1900-1966) whilst a student in Adelaide and she joined him in London where they were married. She had a medical education and worked with the Oxford Regional Blood Transfusion Service from 1939-41. She supervised clinical trials of penicillin conducted at the Radcliffe Infirmary, at military hospitals and at the Birmingham Accident Hospital.

Margaret Jennings

Margaret Jennings

After Ethel's death in 1966, Florey married his research assistant, Margaret Jennings (1904-1994) who had worked on various aspects of penicillin including its toxicology.

Dorothy Hodgkin

Dorothy Crowfoot Hodgkin

The search for the chemical identity of the molecule itself brought other Oxford scientists into the group contributing to the understanding of penicillin. E.P. Abraham in the Dunn School had proposed the structure involving a β-lactam ring, but this had been disputed. In the sub-department of chemical crystallography, Dorothy Crowfoot Hodgkin and her colleagues established the atomic structure of penicillin by means of X-ray crystallography, once a crystallized salt had been produced in 1943. Their findings, made in 1945 and published four years later, confirmed E. P. Abraham's proposal to be correct. The 1964 Nobel Prize in Chemistry was awarded to Dorothy Crowfoot Hodgkin 'for her determinations by X-ray techniques of the structures of important biochemical substances'.

Mary Hunt

The penicillin story moved from Oxford to the USA during the war as the US government and pharmaceutical companies became involved after the Oxford team's visits. Much development work was done at the US Department of Agriculture's Northern Regional Research Laboratory (NRRL) in Peoria, Illinois. Mary Hunt worked at the laboratory and searched for mould strains which might produce more penicillin than Fleming's *Penicillium notatum*. In 1943, she found a mouldy cantaloupe melon in a grocery store. Bringing it to the lab she found it was infected with a different species, *Penicillium chrysogenum*, which produced many times the amount of penicillin. This strain then formed the basis for genetic development and mass production of penicillin, securing an impressive legacy for women in the development of the first antibiotic.

HISTORICAL PERSPECTIVE

Penicillin: The 75th Anniversary of the Nobel Prize

Keith Gull

Penicillin – the antibacterial activity in the extract of the culture supernatant of the *Penicillium notatum* fungus - was discovered by Alexander Fleming at St Mary's Hospital in London (now part of Imperial College) in the late 1920s. The discovery languished undeveloped until Howard Florey, Ernst Chain and the team at the Sir William Dunn School of Pathology in Oxford decided to study antibacterial substances. The Oxford group's work to understand the microbiology and biochemistry of penicillin and to develop it as a therapeutic agent moved at an astonishing pace. It formed the starting point of the antibiotic revolution and produced a paradigm shift in the treatment of infectious disease. As we currently experience an infectious disease pandemic, autumn 2020 marked the 75th anniversary of the award of the 1945 Nobel Prize for Medicine or Physiology to Alexander Fleming, Howard Florey and Ernst Chain (Figure 1). The citation read: 'for the discovery of penicillin and its curative effects in various infectious diseases'.

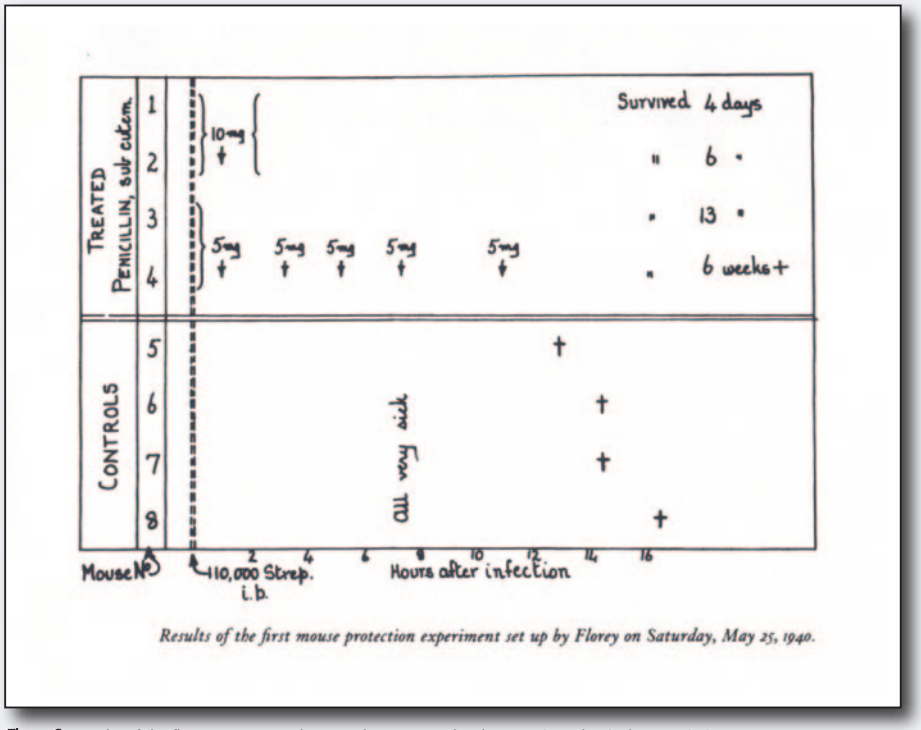
Figure 1. Winners of the Nobel Prize for Physiology or Medicine 1945

Fleming's mould-contaminated petri dish with the zone of inhibition of the bacterial colonies is well known. He observed the importance of the phenomenon and preserved the culture of the fungus. Fleming showed that extracts of the *Penicillium* mould inhibited the growth of a number of important agents of sepsis. He further showed that it did not damage leukocytes and even injected it into a healthy animal, pronouncing that it did no harm. Unaccountably, he never took the step of a systematic study of its effect on infections in animals. Fleming published his findings on the *Penicillium* activity in 1929. His, and others, efforts to purify the unstable active compound from the culture supernatant proved unsuccessful and Fleming's interest in penicillin waned.

The historical context is important. At the start of the 20th century Paul Ehrlich developed the idea of a chemical that would kill an infectious microorganism but leave the host unaffected - the concept of the 'magic bullet'. Ehrlich also understood the importance of a diverse research team dedicated to 'planned chemical synthesis: proceeding from a chemical substance with recognizable activity, making derivatives from it, and then trying each to discover the degree of its activity and effectiveness'. Ehrlich's team introduced of the organoarsenic drug Salvarsan which revolutionized syphilis treatment and established the concept of chemotherapy.

It was, therefore, some decades after the concepts of the magic bullet and chemotherapy were established that, in 1939, Chain and Florey began work on penicillin as part of a comprehensive program of research on antibacterial substances. Henry Harris has commented that 'the choice of penicillin, for Chain, was mainly determined by the challenge posed by its instability, and, for Florey, by the fact that it was the only substance of those considered that was active against *Staphylococci*'. The work progressed well, Chain not only extracted material that had antibacterial activity from the culture supernatant but also established, with J. M. Barnes, that it was not toxic on injection into mice. However, unlike Fleming, the Oxford team had a focus on studying the chemotherapeutic potential of penicillin on infections in animals. On Saturday 25th May 1940, after much innovative work by Norman Heatley in expanding the growth of *Penicillium* cultures, enough penicillin was available for Florey to set up a mouse protection experiment. Heatley, who observed the mice overnight, described the experiment thus: 'eight mice were each given an intraperitoneal injection of virulent *Streptococci*. One hour later, two were given, subcutaneously, a single dose of ten milligrams of a certain penicillin preparation. Two others were given five milligrams then and four further doses, each of five milligrams, at 3, 5, 7 and 11 hours after infection. The other four mice, the controls, received no penicillin. About 7 hours later the controls looked very sick and died between 13 and 17 hours after infection. All the treated mice looked relatively well. Those receiving the single dose survived for four and six days, while of those receiving the larger, divided dose one died after six days and the other remained well until killed some weeks later'. This astonishing and historic experiment was described by Heatley as 'a good example of the kind of simple, well-planned experiment giving clear-cut results which appealed to Florey' (Figure 2).

Florey recognized that, to be successful, the Oxford group needed to be multi-disciplinary. He no doubt drew experience from his previous diverse clinical and laboratory positions in Adelaide, Cambridge, Oxford and Sheffield. Florey understood that biochemical expertise would be a key factor in the purification and identification of the penicillin molecule. He had worked with the brilliant biochemist Albert Szent-Gyorgi in Cambridge in 1929, who advised him that a



Results of the first mouse protection experiment set up by Florey on Saturday, May 25, 1940.

Figure 2. Results of the first mouse protection experiment set up by Florey on Saturday 25th May 1940.

naturally-occurring bioactive agent could be purified from extracts if rapid assays were available. However, in addition to Chain's biochemical skills, the Oxford team had microbiological, chemical, pharmacological, toxicological and clinical expertise. Over the following months, progress was made on increasing the availability of penicillin by improvements in both the growth of the *Penicillium* mould and the purification procedures. The animal infection studies were repeated and expanded to include other bacteria. The results of these studies were published in a ground-breaking paper in the *Lancet* in August of 1940 by Florey, Chain, Abraham, Heatley and other members of the Oxford team. In February 1941 - only some eight months after the initial animal experiments - Florey, along with Charles Fletcher, treated the first patients in the Radcliffe Infirmary in Oxford. Despite some early setbacks and a lack of fully purified penicillin, the results were stunning and revolutionized the treatment of bacterial infectious disease.

The work to elucidate the chemical nature of the penicillin molecule went on alongside these other studies and E. P. Abraham had joined Chain in this effort in the Dunn School. As Henry Harris wrote: 'Abraham, who had recently completed his doctorate in the Department of Organic Chemistry at Oxford (the Dyson Perrins Laboratory) set about the difficult task of purifying penicillin and then determining its structure. Abraham was eventually completely successful in both these aims and was the first to propose the correct chemical structure for penicillin. Abraham's structure, which involved the novel beta-lactam ring, was not accepted by Robert Robinson, the Head of the Dyson Perrins Laboratory or by J. W. Cornforth, then also working in that department; they proposed a thiazolidine-oxazolone structure. This matter was settled by Dorothy Crowfoot (later Hodgkin) who examined crystals provided by Abraham and confirmed by crystallographic methods the presence of the beta-lactam ring.

The pharmaceutical and commercial development of penicillin production moved very quickly in this wartime period and the Oxford team became central figures in that process, travelling initially to the USA in 1941 to advise pharmaceutical company scientists. As the end of the war approached, rumours began of recognition through the award of the Nobel Prize and indeed the 1945 Nobel Prize for Medicine or Physiology was awarded to Howard Florey, Ernst Chain and Alexander Fleming.

It is worth reading the three Nobel Lectures since, in many ways, they perhaps reflect the characters of the men.

Florey's lecture focused on how 'appropriate methods and their coordination' were underpinning the progress in antibiotic research. In essence, he was looking ahead to a forthcoming antibiotic revolution. He ends the lecture with a view of the nature of science and his vision that this intensity of study 'must result in a great accumulation of knowledge, some of which will be immediately applicable to medicine and some of which will contribute to theoretical knowledge. In any event those engaged in the work can look forward to many happy hours of investigation.'

Chain's lecture is entitled 'The chemical structure of the penicillins'. Chain is very precise about acknowledging colleagues in Oxford and around the world who contributed to the work and then proceeds with an academic description of the purification of penicillin and the chemistry of its Beta-lactam ring structure. He notes that definitive proof had lately come from the X-ray crystallographic work of Dorothy Crowfoot Hodgkin and Mrs Rogers-Low in Oxford.

Fleming's lecture is an intriguing, rather historical, backward-looking narrative and contains anecdotal passages seeking to explain why he never used the penicillin extract in experimental infections or in a serious therapeutic study.

Antibiotic research continued in the Dunn School and E.P. Abraham along with Guy Newton, discovered the important antibiotic cephalosporin. In this case, patent income was secured and enabled the establishment of several important and influential charitable trusts that have gone on to benefit biomedical research, education and scholarship.

Finally, at this moment when the world is experiencing the Covid-19 pandemic and science and politics are at the front of the news, it is salutary to rehearse the text of Sir Howard Florey's speech at the Nobel Banquet in Stockholm, on 10th December 1945:

'*Your Royal Highnesses, Your Excellencies, Ladies and Gentlemen,*

I should wish in the first place to thank most sincerely the Nobel Foundation and the Committee for Physiology and Medicine for the very great honour you have conferred on me today. My colleagues and I have been very fortunate in that we have worked during the last few years on something which has proved to be of some immediate value to mankind. During this work I have had the great pleasure of meeting many hundreds of scientific colleagues in many parts of the world. Apart from the scientific interest attached to my various journeyings it has been made clear to me that human needs and aspirations differ little the world over and that no great difficulties arise in one race dealing with another when matters of scientific importance are involved. Thus, on a personal plane, science can act as a force to bring people together, but no-one can, I think, be optimistic at the present time about civilisation as we know it. During the last few years, the demonstration of what the application of scientific methods can achieve has been so striking and of such a magnitude that even those brought up in the classical tradition, who form most of the statesmen and politicians of the world, are at last aware of the tremendous tasks that lie ahead in the utilisation of these forces. We have been astonished at the reaction of some of them to this realisation. Apparently, their idea is that they will utilise and control scientists but that we are so ignorant and insensitive that our views on policies to be pursued and the use to which our work is to be put are of little or no importance. This doctrine I am happy to say has had powerful voices raised against it both in England and America. These voices insist that we must be free to pursue scientific enquiries without political interference. Perhaps on those who have today and in former years received the greatest of distinctions in being awarded a Nobel Prize now rests not only the responsibility for furthering the immediate interests of science but also that of ensuring that those who control our destinies are fully informed of the tremendous forces with which they deal. I feel we must all exert ourselves to the utmost to see that the ideals and hopes held by Alfred Nobel, whom we commemorate today, do not fail from lack of purpose on the part of scientists.

Let us all fervently hope that what can be achieved in the way of friendship on the personal plane among scientists may soon be translated to wider spheres so that the great technical achievements of mankind can indeed be used for its benefit.'

Suggested reading and viewing:

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Photograph Judie Waldmann

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From the *Fusion* Archives...

The following is a selection of excerpts, chosen by the Editor from past editions of *Fusion*, that give a flavour of events and topical issues within the Dunn School during years gone by...

Five years ago...
Steve Cobbold, who was directly involved in the development of CAMPATH-1 (now known as Lemtrada), describes his experiences of receiving a kidney transplant together with the very drug he helped to create [*Fusion* (2016) **15**:16-17]:

The first thing I remember as I awoke in the recovery room was a nurse saying "the CAPMPATH is going in now"! … I have perhaps done the ultimate in clinical translation.

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Irving Weissman recalls, with fondness, the time he spent at the Dunn School working with Jim Gowans [*Fusion* (2016) 15:20]:

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.

Ten years ago...
Upon his retirement, Mike Simpkins recalls the etiquette evident at the Dunn School during the early 1970s [*Fusion* (2011) **10**:14]:

Most senior academic staff wore shirt and tie and usually a tweed jacket with Oxford brogues. In fact, the afternoon tea club, which was exclusive to senior academic staff, required a jacket to be worn on all except the hottest days of the year. Technical staff, on the other hand, quite literally took their tea at the bench.

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Sally Cowley writes about the establishment of the James Martin Stem Cell Facility [*Fusion* (2011) **10**:9-10]:

Using hESCs and hiPSCs, and their differentiated progeny as models to study human disease, is the central aim of the James Martin Stem Cell Facility. The Facility was established… to address a growing need within Oxford for human pluripotent stem cell expertise.

Fifteen years ago...
David Greaves sums up the impact that Siamon Gordon has had on the study of macrophage biology in his report of the symposium held in his honour [*Fusion* (2006) **5**:4-5]:

The Gordon Lab Reunion Symposium… served to remind us of Siamon's many important contributions to the study of innate immunity, not least of which is the international network of scientists and clinicians who continue to study the cells and molecules they first encountered under Siamon's tutelage here in the Dunn School.

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David Wiseman reflects on the publication in *Nature* of the size exclusion model of T cell activation by the van der Merwe group [*Fusion* (2006) **5**:10]:

What we have shown is a whole new way that T cells can control themselves… and all it comes down to is whopping great molecules being shut out of close contact zones…

And finally...

It seems hard to comprehend that it was 26 years ago that I first came to the Dunn School from Cambridge as a post-doctoral fellow in Herman Waldmann's laboratory. As I try to fathom where the time could possibly have gone, I am reminded of the many extraordinary experiences and opportunities I have had over the years and the many poignant memories I shall always treasure. One of the highlights is undoubtedly serving as Editor of *Fusion* for the past 15 years, during which the magazine has evolved in its content and style to reflect, what I hope, is the true spirit of the Dunn School. It has been an enormous privilege to work with so many exceptional people, and I am indebted to everyone who has contributed to its success by providing material for publication and feedback to help it improve. But as my time at the Dunn School draws to a close, I would like to thank all my colleagues, past and present, for their support and friendship over the years. Naturally, I look forward to following the fortunes of the Dunn School in the future, perhaps through the pages of *Fusion*!

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