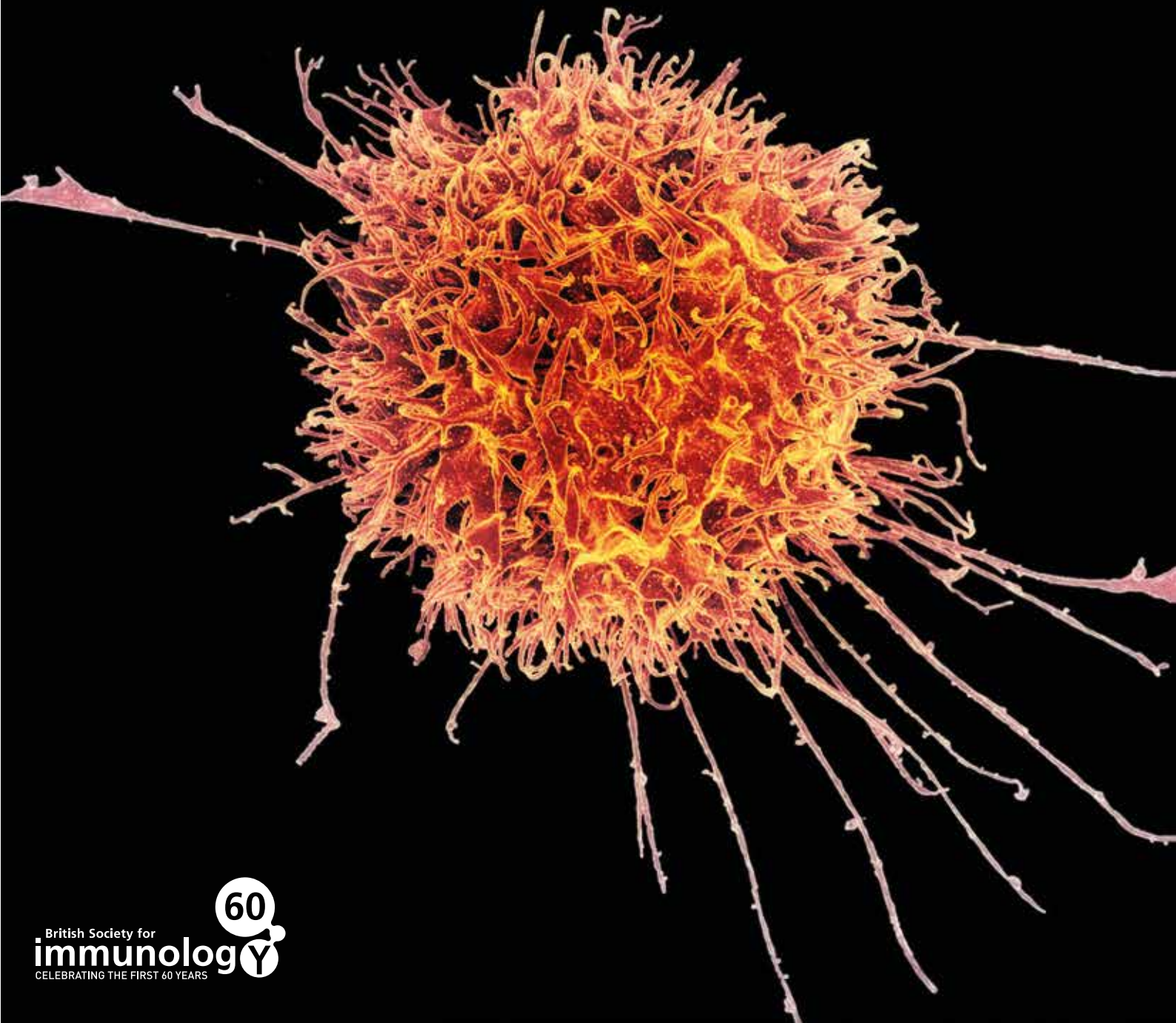
British Society for Immunology | October 2016

60 years of immunology:

past, present and future



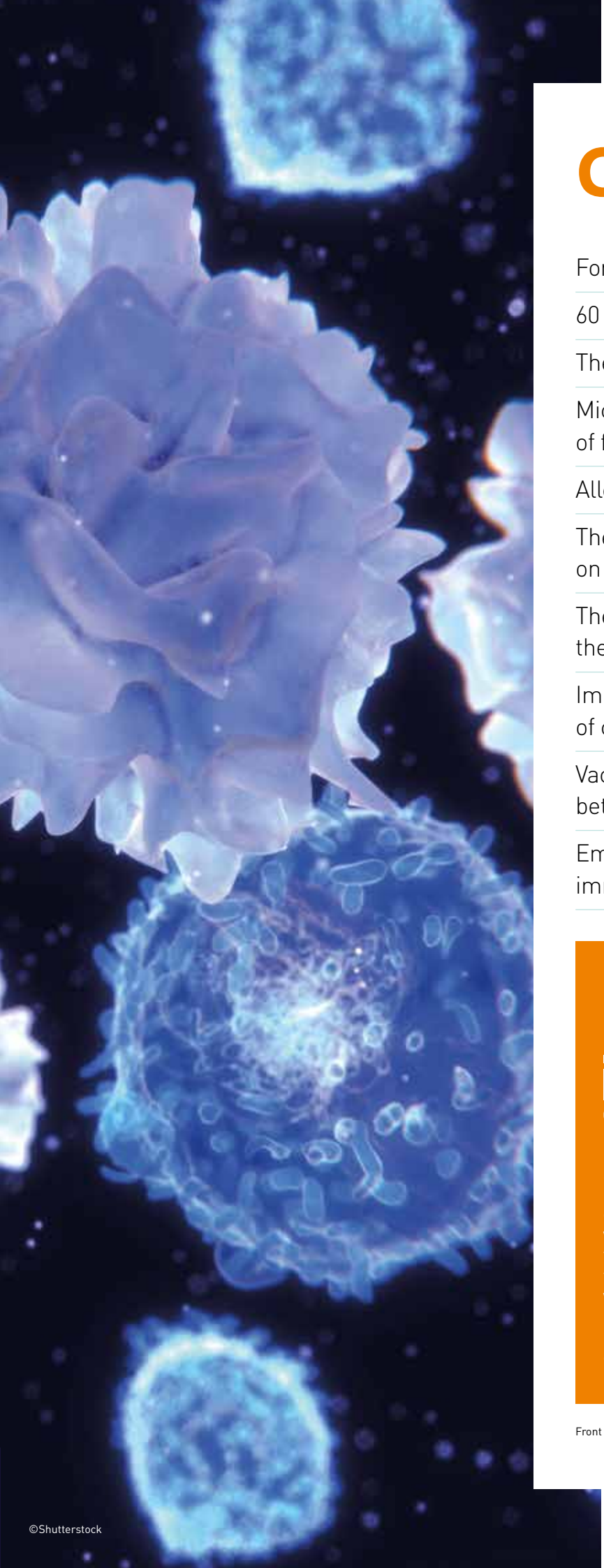
British Society for immunology
CELEBRATING THE FIRST 60 YEARS

Miltenyi Biotec

Miltenyi Biotec is a proud member of the British Society for Immunology.

Immunology has come so far in the last 60 years and it is wonderful to be part of such an inspiring community, working together to understand immunology resulting in significant improvements to both human and animal health.

We look forward to seeing what we can achieve together in the next 60 years!



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British Society for immunology 60

CELEBRATING THE FIRST 60 YEARS

2016 marks the 60th anniversary of the British Society for Immunology. The Society was founded in 1956 by a small group of hard working, visionary immunologists, who wanted to come together to share ideas and encourage the study of immunology. Today, the British Society for Immunology has over 3,000 members worldwide who support our mission to promote excellence in immunological research, scholarship and clinical practice in order to improve human and animal health. To find out more about our work, visit **www.immunology.org**.

Front cover image: Human Natural Killer Cell © NIAID/Flickr CC-BY-NC 2.0

Foreword

British Society for Immunology at 60

To quote Peter Medawar, Nobel Prize winner and a father of modern immunology, *"Scientific reasoning is a kind of dialogue between the possible and the actual, between what might be and what is in fact the case."* Since Sir Peter's time, the British Society for Immunology (BSI) has been at the forefront of unravelling the intricacies behind this dialogue, resolving fact from speculation. The immune system, both in humans and animals, has proved to be immensely more elegant and complex than ever could have been imagined when the BSI was established. Its nature has truly proven fundamental to the way we are made and to how we interact with our environment.

The BSI is Europe's largest and oldest society dedicated to supporting immunology and immunologists. Founded 60 years ago, we are fortunate in having a substantial income from our journals, *Immunology* and *Clinical & Experimental Immunology*; this allows us to do many things for our members that would not otherwise be possible.

The purpose of this monograph is to celebrate the BSI's first 60 years by highlighting selected areas of current immunological interest and identifying exciting new themes and trends. The review is unashamedly UK-centric but also emphasises the contribution that overseas nationals have made to UK immunology in addition to that made by British immunologists who have emigrated to all corners of the globe in order to pursue their diverse research interests. With the recent decision of the UK to leave the European Union, it is especially opportune to highlight the compelling internationalism of our discipline.

My own career as an immunologist was driven by the puzzling patients that I encountered during my medical training, in particular when I was on the wards looking after renal patients at Guy's Hospital and subsequently caring for patients with inflammatory lung diseases at the Royal Brompton Hospital. I was originally determined to be a physiologist, but seeing these patients with ill-understood immunological conditions that mounted a relentless attack on their vital organs drove me to the conclusion that immunology was something I needed to know more about.

In the mid-1980s we indeed knew a bit about T cells, quite a lot about antibodies, but very little about how inflammation was triggered and perpetuated. I remember Margaret Turner-Warwick (a thoracic medicine specialist and later President of the Royal College of Physicians), at the end of a particularly complex and difficult case presentation, shrugging her shoulders and saying, "Clearly, we have another case of immunological mischief!" That was about as far as we could get at that point in time.

Now, in 2016, both basic and applied immunology is undergoing a revolution. Almost every week there is a remarkable advance in understanding of the basic science behind how the immune systems functions, and month by month new treatments are licensed that extend the therapeutic options we are able to offer patients. There is an extraordinary range of immune modifiers going into clinical trial, no longer just steroids and general immunosuppressants. As the options for immunological therapy are explored, results that were unanticipated from animal models tell us more about how the immune system works. As we gather knowledge and experience, vaccines and immune treatments are set to bring immense benefits around the globe (if only we can afford them).

At a more fundamental level, the revelations produced by immunological research over the past 60 years have been quite astonishing. A large part of the mammalian genome is devoted to the immunological arms race against commensals and pathogens, each discovery revealing new depths and unexpected intricacies. We have certainly not yet developed a full picture of how the immune system works, each discovery revealing new levels of ignorance that need to be explored in future experiments.

From my personal viewpoint, the evolution of the immune system and its actions have to be understood in the context of the pathogens and commensals with which we have co-existed throughout history. Bugs are arguably the world's best immunologists: they know and control the immune system from the inside out; their very survival depends on their inbuilt databank of immunological wisdom.

As my PhD mentor Brigitte 'Ita' Askonas (1923–2013) often remarked, it is hard to imagine how little we knew about the immune system when the BSI was first established. In my own lifetime, the era of animal experimentation has been fundamental in informing us about how the immune system operates. However, ultimately we have to perform experimental studies in humans to test these ideas and to develop targeted therapies tailor-made to the disease and the individual.

The rich pipeline of biological agents now being developed by major pharmaceutical companies is a remarkable testimony to the extraordinary productivity of immunologists. Many immunological advances depend on parallel developments in other fields such as chemistry, physics, optics, microscopy and genomics. The opportunities for immunologists to innovate in partnership with these other sciences continues to be as exciting as ever: progress in treating cancer, neurology, transplant, rejection and a whole plethora of immunological diseases is leaping forward year by year because of these collaborations.

The revolution that has come about based on extensive and intricate animal work is now often combined with 'big data' projects comparing the results of animal and human studies. Although human experiments are revealing, they are usually highly reliant on using the patient's blood as a source of immune cells to measure effectiveness, whereas the real immunological action and effect happens in tissues that are inaccessible or hard to reach by conventional techniques. Again, new methods of monitoring local inflammation at mucosal or other sites are set to create whole new fields of opportunity for the discipline in the future.

However, we still have so much to learn and many questions are as yet unanswered. For example, vaccination may induce a change that we can measure, but is this actually what causes the protection offered by the vaccine? Are the so-called correlates of protection just correlating with something else that is really doing the protecting? Do the biological response modifiers that we want to test in clinical conditions work the way that we think they do, or are they functioning in some other mysterious way yet to be determined? If we find something is overactive or elevated in a disease condition, does this mean that the overactivity should be blocked, or should we enhance that activity because it is part of the healing process? The vaccinations schedule that we give to our children has expanded from covering two infectious diseases when the NHS was launched in 1948 to 20 infectious diseases in 2016. In the future, how can we give the same protection to children, but with fewer needles?

The BSI is proud to have supported immunology and immunologists throughout 60 very exciting years. We look forward to working with our members, with those who fund immunology and with key opinion leaders in government and industry, to build on all these successes. The BSI is more than just British: we are pleased to support our overseas members and to develop the international collaborations on which the best science depends. No single lab, no single nation or continent contains the know-how to solve the many pressing issues that continue to puzzle immunologists and clinicians.

Our discipline is global. The BSI will continue to play a vital role in sustaining and expanding immunology's future, and in doing so we are acutely conscious of the need to continually innovate and advance. As the Red Queen in Lewis Carroll's *Through the Looking Glass* said, "...it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!"

The BSI is here to help immunologists become equipped with the best running shoes there are.

Peter Openshaw
President, British Society for Immunology

1956
British Society for Immunology formed

1959


Structure of antibodies determined by Rodney Porter & Gerald Edelman

1960


Nobel Prize in Physiology or Medicine awarded to Peter Medawar & Frank Macfarlane Burnet for the discovery of acquired immunological tolerance

1956

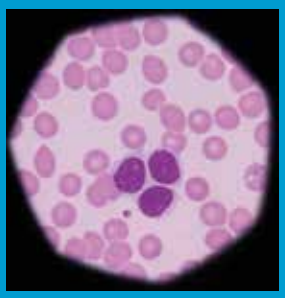
1960

1956
First human polio vaccine licensed for use in UK

1957
Rheumatoid arthritis found to be an autoimmune disease

1968
First successful human bone marrow transplant

1966
Lymphocytes determined to be the source of immunological memory



1970

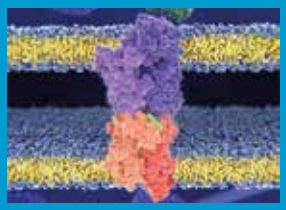
1969
Helper T cells first discovered

1973
Dendritic cells first discovered

1975
First monoclonal antibodies produced

1976
Genetic mechanism that produces antibody diversity discovered

1981
Macrophage antigen presentation to T cells shown



1980

1980


World Health Organization declares that smallpox has officially been eradicated

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60 years of immunology

2015

Confirmed link between brain and immune system

2016

2010

2013

Cancer immunotherapy named 'Breakthrough of the Year' by *Science*

2011

United Nations declare that rinderpest in cattle has officially been eradicated

2014

Ebola outbreak in West Africa ushers in the need for global collaboration to quickly develop and manufacture effective therapeutics

2001

Alemtuzumab, the first monoclonal antibody against cancer, is launched under the trade name Campath

2000

2001

The term 'microbiome' first coined

1989

Hygiene hypothesis put forward

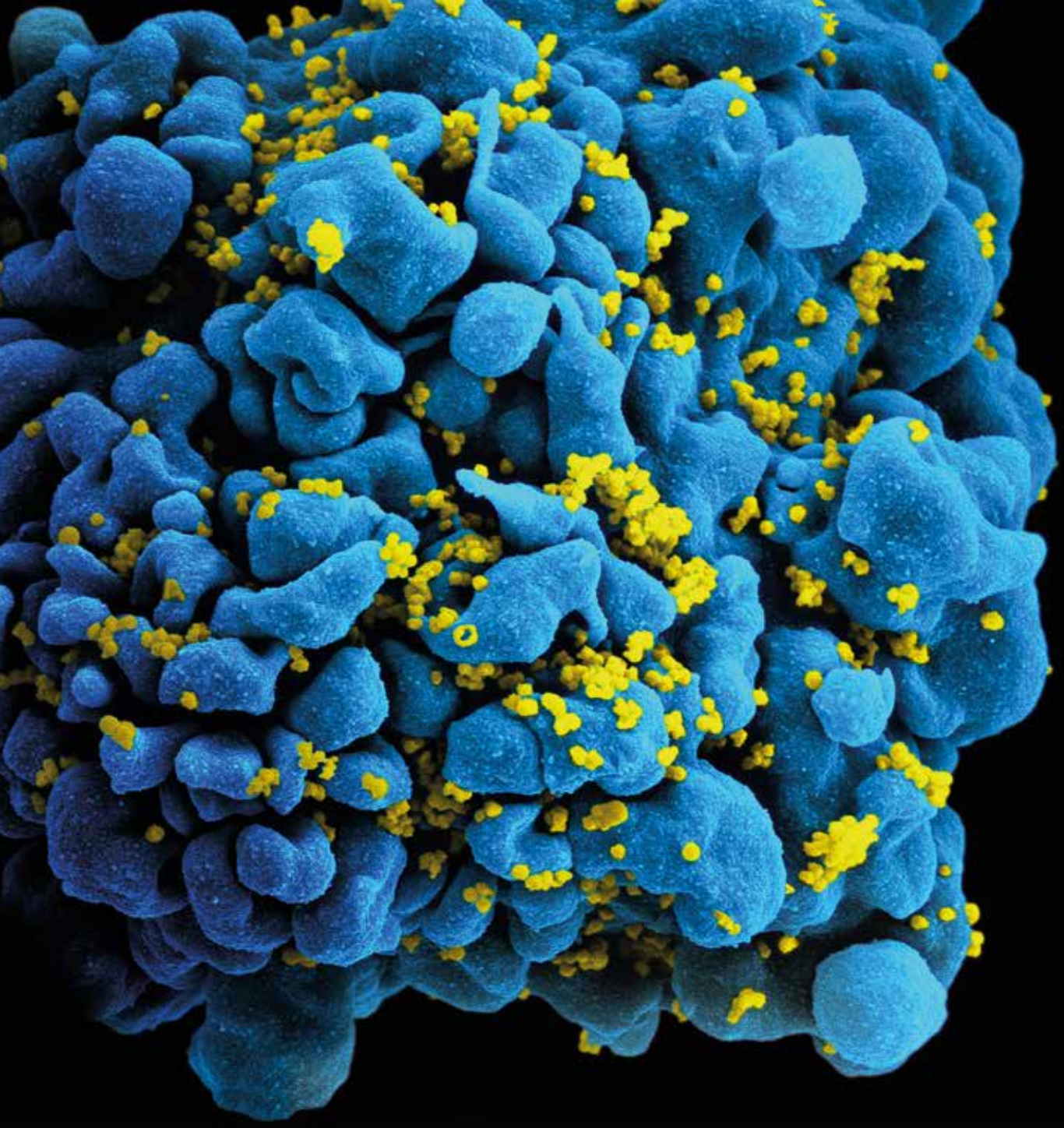
1990

1986

First vaccine produced by genetic engineering approved for human use to treat hepatitis B

1993

First successful clinical use of anti-TNF monoclonal antibodies to treat rheumatoid arthritis



The hunt for an HIV vaccine

On 23 April 1984, Margaret Heckler, the then US Secretary of Health and Human Services, announced to a packed press conference that scientists had discovered the virus that caused acquired immune deficiency syndrome (AIDS). She went on to express the hope that a vaccine would be developed within two years.

HIV virus infected T cell. © NIAID

A doctor examines a blood sample to find out if it is positive for HIV

Thirty-two years later and AIDS-related illnesses have claimed at least 30 million lives. In 2015, around 2.1 million people were newly infected with the virus and approximately 1.1 million people died as a result of the disease. Globally, the rate of infections and deaths have declined over the years thanks to the use of antiretroviral therapy and reduced spread of the human immunodeficiency virus (HIV) responsible for the disease; however the availability of treatment and impact of the disease vary widely across the world. It is widely acknowledged that the most effective way to end the HIV pandemic would be to develop and roll out an effective protective vaccine.

Biggest biomedical challenge of our generation

Even at the time, immunologists who heard Heckler's infamous prediction about how long it would take to develop an HIV vaccine knew it to be wildly optimistic. More than three decades on, much more is known about the myriad of devious ways through which HIV evades the body's natural defences and the scale of the task faced by those trying to beat it.

For a start, because no-one has ever fully cured themselves of HIV infection, researchers cannot simply imitate the immune responses of those who have spontaneously recovered, as they have done with many other infections. HIV is highly genetically variable, both replicating and mutating more rapidly than many other viruses. On top of this, the virus is surrounded by a dense coat of sugars that stop immune system antibodies locking on and identifying it as an enemy. "It's one of the biggest biomedical challenges of our generation," says Professor Robin Shattock of Imperial College London.

Early disappointment

The hunt for an effective HIV vaccine has come in three phases. The high point of the first phase, based on using simple viral proteins to induce antibodies with the aim of disabling the virus, came with the launch of trials of AIDSVAX in 1998 and 1999. This was the first HIV vaccine to enter full-scale efficacy testing. Disappointingly no evidence of protection was found and the AIDSVAX trials ended in 2003.

The discovery around 1990 that CD8 killer T cells play a key role in controlling HIV shortly after infection by killing infected cells led scientists to wonder whether a similar response could be induced by vaccination before infection. The high point for this second phase in the hunt for a vaccine came with the launch of the STEP trial of an adenovirus (Ad5) vector vaccine encoding three synthetic HIV genes in 2004. Again optimism turned to disappointment in 2007 when it became clear the candidate vaccine neither prevented HIV infection nor reduced the amount of virus in those already infected.

A helping hand

One of the characteristics that makes HIV hard to combat is its ability to take over a host's CD4 helper T cells and then turn them into factories to generate copies of itself. The virus accesses the helper T cells via a surface glycoprotein called CD4 and co-receptors CCR5 and CXCR4. The identification of these mechanisms was of fundamental importance in narrowing the focus of those working on HIV.

Dr Daniel Douek's group at the National Institute of Allergy and Infectious Diseases at the National Institutes of Health in Bethesda, USA, showed in 2004 that HIV infection causes the rapid depletion of the majority of these CD4 T cells in the gut, and that levels don't recover. Douek and colleagues later showed HIV infection can cause damage to the gut barrier, thereby allowing microbes to cross it. Douek says this helps explain the systemic immune system activation known to be a key driving force in HIV disease progression.

These insights have led Douek (who originally trained in the UK before moving to the States) to look more closely at the role of the mucosal tissue that lines cavities in the body and surrounds internal organs. Most recently, he has been looking at the importance of lymph nodes as sites of HIV replication and maintenance. Shattock and others have shown that it is predominantly CD4 T cells in the mucosa that are the first cells to be infected. He and others are investigating whether boosting immune responses in the mucosa could stop the virus in its tracks.

> *"Many scientists now believe that rather than deploying a single silver bullet against HIV, the best hopes of success in the hunt for an effective vaccine lie in a combined assault"*

Doctor and patient at an HIV clinic

A new approach

The failure of the STEP trial in 2007 led many in the field to move away from efforts to produce vaccines by stimulating killer T cell activity. However, recently there has been renewed confidence in such an approach since 2013 when it was reported that nine of 16 rhesus monkeys given a vaccine and then infected with simian immunodeficiency virus (SIV), a close relative of HIV, were able to completely clear the virus. The vaccine is based on a form of cytomegalovirus (CMV), a common member of the herpes virus family, which had been modified to include SIV genes. It works by training the immune system to recognise and attack SIV infected cells.

Professor Louis Picker of the Vaccine and Gene Therapy Institute at Oregon Health and Science University in Beaverton, Oregon, who leads the CMV work, has carried out other animal trials but has been unable to achieve protection levels above 50–60%. He believes that this could be because SIV is more virulent than HIV, and that higher levels of efficacy may be possible in humans. CMV is thought to be carried by 50–80% of adults in the UK, and in most cases doesn't cause any obvious symptoms.

Picker is, of course, aware that previous approaches to tackling HIV that have shown promise in animals have later failed to have an effect in humans. Nonetheless, recruitment of around 75 people for safety trials of an attenuated human CMV-based HIV vaccine began in June.

Neutralising HIV variability

Another of HIV's trump cards is its ability to evade detection by immune system antibodies through its sheer variability. Around the same time as researchers were dissecting the disappointing AIDSVAX and STEP results, it was discovered that a small percentage of infected patients, known as 'elite controllers', produced broadly neutralising antibodies (bNAbs) capable of acting against multiple strains of HIV-1 (the most common type of HIV). Although the virus in these subjects was already resistant to these antibodies, the finding demonstrated that humans are capable of producing them in response to natural infection and stimulated efforts to design vaccines based on triggering the production of bNAbs. "We know these antibodies can be made in infected humans," says Shattock. "The question of how we get non-infected individuals to make them as a preventative vaccine has fuelled a whole raft of advanced immunological research."

Those seeking to answer this question include a team led by Professor Michel C. Nussenzweig at the Rockefeller University in New York. Last year his group published evidence that a cloned bNAb called 3BNC117 significantly reduced the amount of HIV in the blood of infected patients. It lost most of its effectiveness within 28 days in some patients, suggesting the virus had mutated to evade detection, however there are hopes that cocktails of bNAbs could overcome resistance.

Meanwhile research published in July by researchers at Duke University in Durham, USA, showed individuals capable of producing high levels of bNAbs against HIV also often have higher levels of antibodies that attack the body's own cells (called autoantibodies), fewer regulatory T cells and higher levels of memory T follicular helper cells. This provides important clues to advance our understanding of the basic biology of bNAb induction and enhances the prospects of future efforts to develop a vaccine based on stimulating their production.

Targeting the envelope spike

Today, scientists seeking to identify bNAbs benefit from recent advances in the fine mapping of the structures on the outside of the virus. Its genetic material and protective protein shell are surrounded by an external envelope that includes glycoproteins, which together form an 'envelope spike' that allows it to bind to and enter host cells.

"The more structural information you have, the better you can design immunogens to mimic what is on the virus spike to induce neutralising antibodies," says Professor John Moore, a British immunologist at Cornell University's medical school in New York.

What makes mimicking the HIV envelope spike difficult is that it undergoes a series of rearrangements to enable it to carry out its functions. In 2013, Moore's group produced the first stabilised recombinant envelope protein compound capable of inducing antibodies against itself. "It was the first time somebody has been able to induce strong neutralisation against HIV," says Shattock.

Lab for HIV vaccine

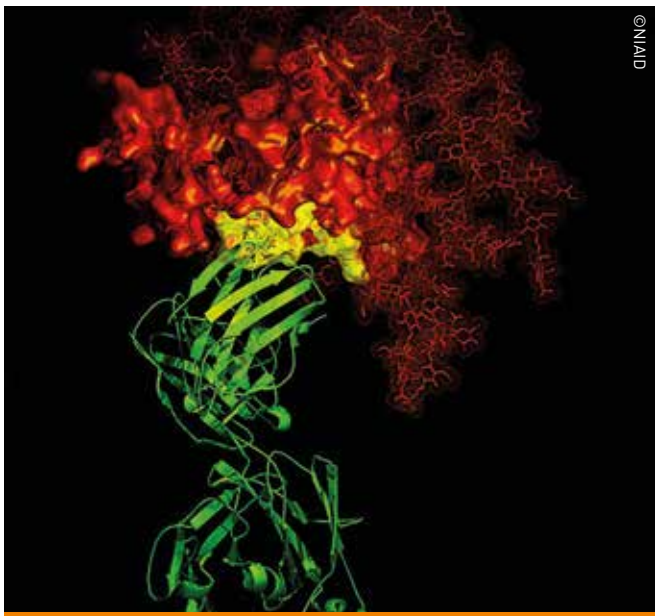
Moore says the advance was aided by the development in recent years of advanced electron microscope technology at the Scripps Research Institute in La Jolla, California, as this allowed his group to properly visualise the proteins they were making. He also warns that there is still a lot of work to be done if their breakthrough is to benefit patients. "The neutralising response we can induce is of narrow specificity, which is of no real value in an HIV vaccine because the virus is so variable. The goal now is to broaden the response and induce bNAbs," he says. "That's not a trivial exercise."

A combined assault

Many scientists now believe that rather than deploying a single silver bullet against HIV, the best hopes of success lie in a combined assault to induce both bNAbs and killer T cells – what many see as the third phase in the hunt for an effective vaccine. This was the approach taken by what has been called the first 'successful' HIV vaccine trial, the results of which were published in 2009. The trial – called RV144 – combined two previous vaccines that had shown no efficacy on their own. The first was AIDSVAX, and the second was ALVAC-HIV, a modified, recombinant canarypox virus that expresses multiple HIV proteins.

Researchers at the US Military HIV Research Program vaccinated one group of Thai volunteers with the vaccine and another control group with a placebo in 2003, and then tested them for HIV over three years ending in 2006. They found that those who received the vaccine were 31% less likely to become infected than those given a placebo.

This level of protection is not high enough to justify its use and opinions have varied as to how much promise the 'Thai trial' approach offers for the future; however efforts are ongoing to find ways to boost the protection RV144 provides. "That was a very important milestone," says Douek. "It's not quite the light at the end of the tunnel, but it showed there is at least a tunnel you can start going down."



3D X-ray crystallographic image showing a broadly neutralising antibody (green ribbon) in contact with a critical target (yellow) on an HIV-1 virus (red).

Patient receives an HIV test

> *"Another of HIV's trump cards is its ability to evade detection by immune system antibodies through its sheer variability"*

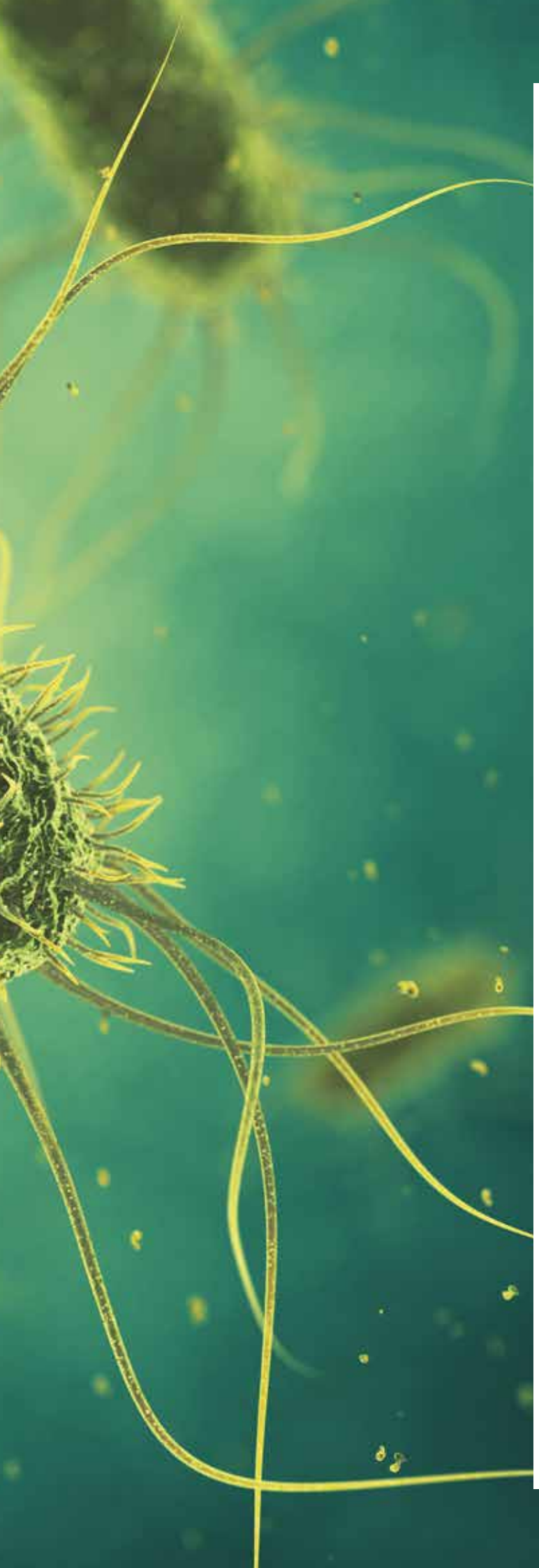
An international problem with an international solution

The combination strategy is also at the heart of the European Aids Vaccine Initiative, launched in October last year to pool the efforts of research groups at 22 institutions in nine EU countries, Australia, Canada and the US to develop new candidate HIV vaccines that can be taken through to human trials within five years. Known as EAVI2020, the collaboration is led by Imperial College London and is backed by €23 million from the European Commission. Some EAVI2020 researchers are working on synthetic envelope vaccines to induce bNAbs, while others are seeking to build on Picker's work, designing vaccines that trigger broad T cell responses by targeting conserved parts of the virus that are unable to mutate. The plan is demonstrate the effectiveness of the best candidates for both approaches in human studies and combine them into one, hopefully effective, vaccine.

Whether the growing hopes that this 'third generation' combined approach to the problem of developing a HIV vaccine will prove to be successful or another false dawn remains to be seen. The repeated dead ends, failures and frustrations that researchers have faced since efforts to find an HIV vaccine began in the 1980s have primed them to be sparing in their optimism. And yet, paradoxically, it is also a characteristic that is practically a pre-requisite for working in the field. "I know a lot of very clever people who are working on this problem, and I know we'll make progress," says Douek. "I don't know how long it will take, but I do know we'll succeed."

Microbiota:
hidden communities of friends and foes

Odd as it might sound, most of the average person isn't human. Researchers have recently dismissed the often cited claim that there are 10 times more microbes than human cells in our bodies. Scientists in Israel who published new estimates in August say the ratio is closer to 4:3 in men and 11:5 in women.



These complex communities of tens of trillions of bacteria and other single-celled organisms that inhabit the gut, lungs, skin and other parts of the body are known collectively as the microbiota. They interact with the parts of our bodies that make up their environment in ways that can be beneficial, neutral or harmful to us. Particular genera or species of microorganisms are described as commensal, symbiotic or pathogenic depending on their known impacts on our health.

Step change in our understanding

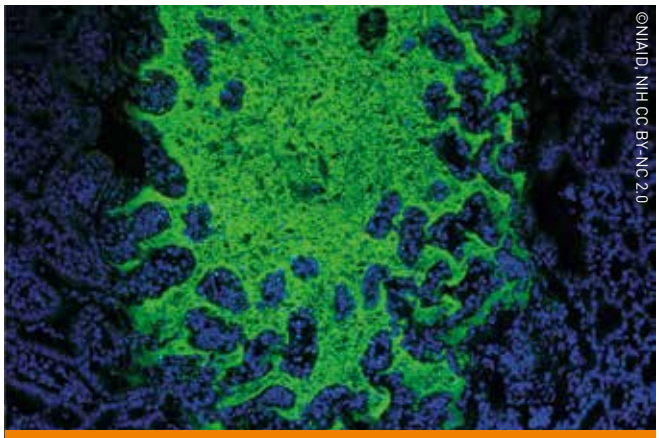
Recent years have seen a rapid growth in our understanding of the many ways these bugs influence our day-to-day functioning. They provide us with nutrients and energy by helping to break down food, and even produce chemicals that can influence our mood. Some believe their combined genomes – known as the microbiome – may exert a greater influence on our health than the genes we inherit from our parents. "There has been a step change in our understanding of the fundamental roles these microbes play in the development and functioning of the immune system in the last decade," says Professor Fiona Powrie, Director of the Kennedy Institute of Rheumatology at the University of Oxford.

Powrie, who carried out pioneering work on defining the roles of regulatory T cells in self-tolerance and autoimmune disease, has also long studied the interactions between the immune system and microbes in the gut. She has shown how specific species of bacteria promote the production of regulatory T cells and effector T cells (those that bring about an immune response), and described details of the roles they play in driving inflammation. "We don't yet understand all of the pathways by which the microbiota influence regulatory T cell differentiation, but we do know that when certain microbes are deficient, those pathways can be diminished," says Powrie.

Lung communities

While Powrie and most other scientists looking at the human microbiota have focused on the gut, others have shown that bacterial communities in different parts of the body have their own distinct characteristics and roles. After carrying out early landmark genetics studies on asthma, Professor Miriam Moffatt and colleague Professor William Cookson, both at Imperial College London, began to investigate the roles for microbes in the lungs and airways. At the time, medical students were taught that the lungs were sterile – something their previous work had led them to doubt.

The emergence and development of molecular techniques has revolutionised microbiology, with the rapid increase in speed and accuracy of genetic analysis tools based on high-throughput sequencing. Moffatt and Cookson used 16S rRNA gene sequencing, a technique previously employed to determine the relationships between organisms. It makes use of the highly conserved nature of the 16S rRNA gene to allow the identification of bacteria and other microbes, although often down to the genera rather than the species level. The Imperial team combined the technique with next-generation sequencing to characterise the microbiota in the airways of healthy people as well as in patients with asthma and chronic obstructive pulmonary disease (COPD).



Visualisation of bacteria in the small intestine of *Toxoplasma gondii*-infected mice. Green indicates bacteria; blue indicates intestinal cells and immune cells.

"We showed that healthy airways are not sterile, but have a characteristic community of bacteria, and also identified specific groups of bacteria that are more abundant in those with asthma and COPD," says Moffatt.

Earlier this year, along with their collaborators, Moffatt and Cookson published a study in which DNA sequencing of sputum from the lower airways identified marked differences in the make-up of the bacterial communities in the lower airways of severe asthmatics, non-severe asthmatics and healthy individuals. Certain species within the *Firmicutes* phyla were correlated with asthma severity and characteristics, for example. Moffatt acknowledges however that the study could not tell them whether the different levels of bacteria were causes of ill health, or the results of it.

Checkpoint blockages

Along with greater understanding of the importance of microbes within the body to immune defences has come an appreciation of their roles in influencing the effectiveness of treatments for disease. Drug regulators have in recent years approved a number of cancer immunotherapy therapies that work by blocking inhibitory signals known as checkpoints that normally act to prevent inappropriate immune responses. These have been shown to be highly effective for some patients but ineffective in others. Scientists have been trying to find out why in order to improve patient outcomes.

A team from the University of Chicago showed last year that tumour growth in mice varies depending on the microbial composition of the gut, and that immune checkpoint blockade therapy became more effective in those prone to melanomas when they were given faeces from less susceptible mice. French scientists have also shown a treatment that acts on checkpoint target CTLA-4, an important regulator of T cell responses, doesn't work in mice that are germ-free or have been treated with antibiotics.

Bacterial gut composition is also believed to explain why around 30% of patients given anti-CTLA-4 cancer immunotherapy get colitis as a side effect. "The pathways involved are not completely understood, but what we are learning is that the outcome of cancer immunotherapy, including both efficacy and side effects, are dependent on aspects of the microbiota," says Powrie, who published a review of research on the impact of gut microbes on cancer immunotherapy in December last year.

Putting knowledge into practice

There is of course a big difference between understanding how the microbiota influence immune responses, and devising interventions to improve our health. As with many promising areas of biomedical science, some of the claims being made in this area, especially those with commercial interests at stake, are not necessarily backed up by the research evidence. Despite this, the value of the worldwide market in probiotics – yoghurts and food supplements containing so-called 'friendly' bacteria and yeasts – stood at $62.7 billion in 2014, according to one recent estimate.

There is evidence that probiotics can reduce the risk of infectious diarrhoea associated with antibiotic use by almost half, and be beneficial to those with ulcerative colitis and pouchitis, a complication of surgical treatment for ulcerative colitis. Faecal microbiota transplants have also been shown to be effective in treating the recurrent hospital-acquired infection *Clostridium difficile*.

The evidence on whether probiotics improve outcomes for those with respiratory infections and colds is mixed. Claims that they can lower blood pressure and cholesterol, help with weight loss, prevent or improve skin conditions, anxiety, depression and urinary tract infections are not supported by strong evidence. Research has shown that those with a variety of different conditions including cardiovascular disease, type 2 diabetes, Alzheimer's and Parkinson's have differences in their microbiota compared to healthy people; however what is not clear is whether this is part of what has caused their ill health or just a consequence of it.

Cause or effect?

"Changes in the microbiota are important in a number of diseases, particularly inflammatory diseases of the gut," says Professor Julian Parkhill, of the Wellcome Trust Sanger Institute. "What we don't really know yet is whether the changes we see in the microbiota are cause or whether they are effect, and that's a very difficult question to answer. I think there's a lot of potential for some specific diseases, but there's been a lot of hype around the microbiota and a lot of things that are currently touted as potential avenues of treatment may well not pan out."

> *"What we don't really know is whether the changes we see in the microbiota are cause or whether they are effect, and that's a very difficult question to answer"*

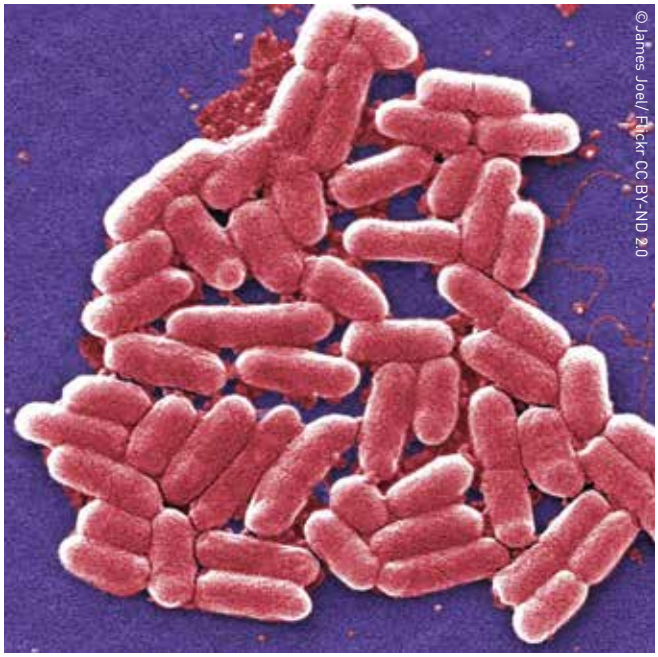
Parkhill, who studies pathogen diversity and variability using high throughput sequencing and phenotyping, says the methods being used by researchers to identify the beneficial and harmful bugs inside us are not yet advanced enough to come to definitive conclusions. "Our understanding of the microbiota is generally at species level, and we really know almost nothing about sub-species diversity, which can be enormous and have big effects in the real world," he says.

Moffatt believes interventions to alter the microbiota that interact with the respiratory system are some way off. "We've still got a lot more to learn," she says. "We have to be very careful because we don't yet fully understand how we interact with our microbiome." Moffatt believes interventions to alter the microbiota that interact with the respiratory system are some way off. "We've still got a lot more to learn," she says. "We have to be very careful because we don't yet fully understand how we interact with our microbiome."

Revolution of molecular techniques

Molecular techniques, such as the 16S rRNA gene sequencing method used by Moffatt, have revolutionised standard microbiology and driven progress on the understanding of the microbiota. Whole microbial genome sequencing offers the comprehensive detail that can unlock an organism's functions and roles, however it is still relatively costly and complex when there are large numbers of organisms to characterise. Advanced selective sequencing techniques are available, but organisms still need to be isolated and grown in the laboratory in order to study them in depth.

One of the major roadblocks to understanding the roles microbes play in the human body has been the idea that the vast majority of the bacteria in our guts cannot be cultured using traditional laboratory methods because they die when exposed to oxygen. "There's this dogma in that 90% of the bacteria of the gut can't be cultured, and that therefore the only way we can address them is bulk sequencing, but the problem with bulk sequencing is you have to know what you're looking for before you can interpret the data," says Parkhill.



> *"The emergence and development of molecular techniques has revolutionised microbiology, with the rapid increase in speed and accuracy of genetic analysis tools based on high-throughput sequencing"*

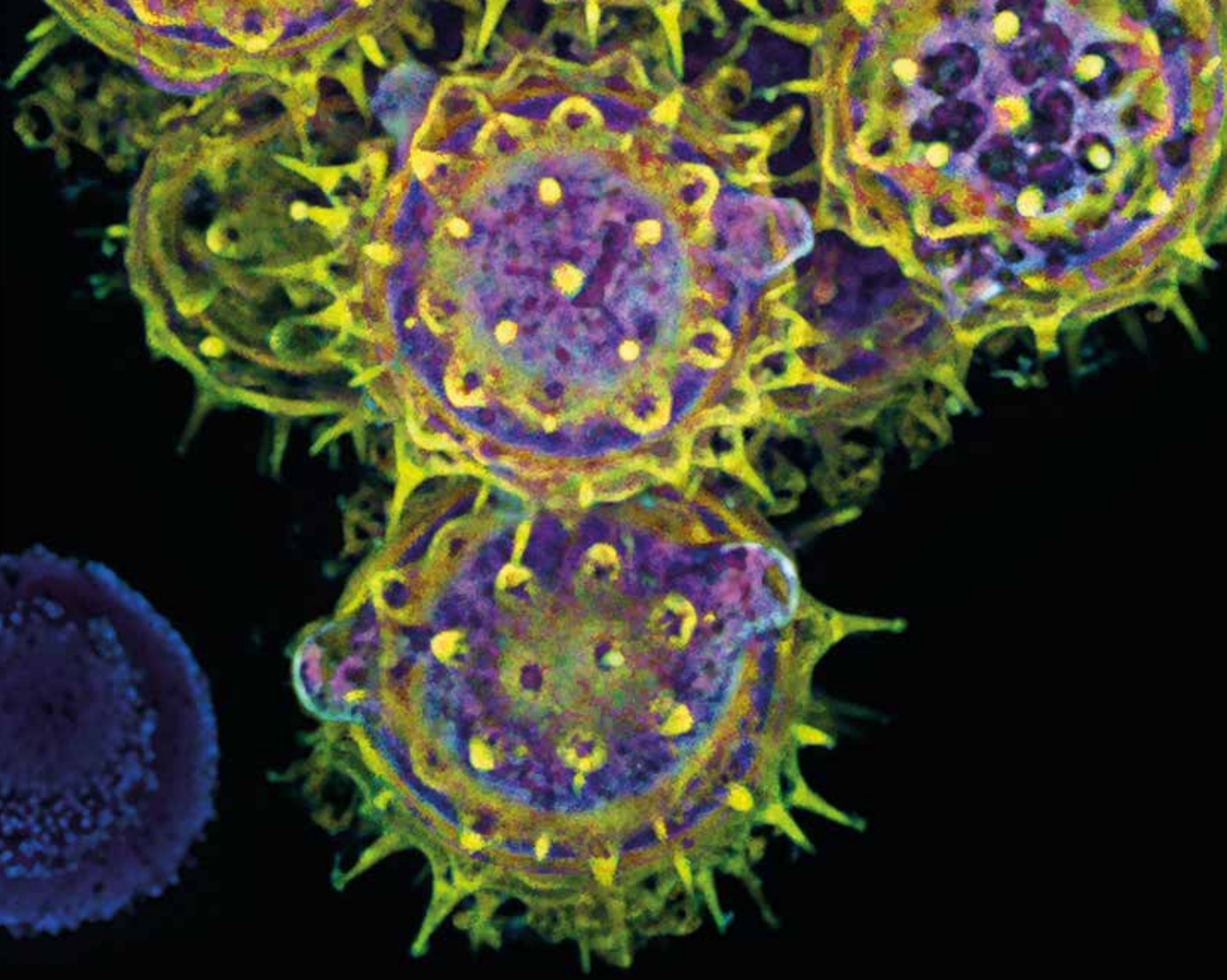
Earlier this year a group led by Professor Trevor Lawley, also at the Sanger Institute, combined large-scale whole genome sequencing, phylogenetic analysis, computational modelling and targeted culturing to show that 50–60% of intestinal microbiota bacteria produce resilient spores that can survive outside the human body. The breakthrough, if confirmed, should allow researchers to culture the microbes that make up most of the gut microbiota and which were previously thought impossible to culture. This of course opens the door to far greater understanding of their roles in the human body.

Beyond the specific identities of the organisms involved, another approach being taken is to focus on their functions and the metabolites they generate that facilitate their roles. "Scientists are starting to scratch the surface of bacterial metabolites and understanding their functions in terms of immune cell activity," says Powrie. It is known that gut bacteria are important in breaking down otherwise undigestible carbohydrates into short-chain fatty acids, which play important roles such as preventing diet-induced obesity and insulin resistance, for example.

Laying the foundations

Many scientists investigating the relationship between the microbiota and the immune system are keenly aware that overblown early claims have led to disappointments in other fields. Yet at the same time, it is hard to avoid the conclusion that the rapid advances in knowledge and experimental techniques of the last 5–10 years can lay the foundations for a host of major health benefits in the not too distant future.

"So many different technologies have come together in the last five years," adds Powrie. "The advances in sequencing and the new approach to culturing components of the microbiota have come alongside advances in gene editing and stem cell technologies. We're poised to bring these cross disciplinary approaches together, and move from model systems to human patients. It really is an exciting time to be working in immunology, and medical research in general."



Allergies:
an inflammatory subject

Whether it's to pollen or peanuts, dander, dust mites or mould, the chances are that you, or someone you know, has an allergy. Nut-free schools are now common, and teachers are often trained in how to administer adrenaline in the case of a pupil suffering a severe allergic reaction. According to figures from the European Academy of Allergy and Clinical Immunology, diagnoses of food allergy have doubled in the past decade, while the number of hospitalisations caused by severe allergic reactions has risen seven-fold. It estimates that, within ten years, more than half of Europeans will be affected by allergy.

Figures such as these have prompted many to conclude that we're in the midst of an allergy epidemic. The reality may be slightly more complex: the prevalence of some allergies, particularly to food, may have been overestimated, while the incidence of others, such as asthma and eczema, may have plateaued, or even slightly declined.[1] But allergies are certainly more prevalent than they were 50–100 years ago, and they also seem to be a peculiarly Western phenomenon.

Competing hypotheses

Why should this be? One popular theory is that the rise in allergies is the price we must pay for increased cleanliness and fewer childhood infections. This so-called 'hygiene hypothesis' of allergic disease was first proposed in 1989, but subsequent studies have refuted the idea that reduced exposure to pathogens is the cause. More likely, it's a lack of exposure to the myriad of microorganisms and parasites that were present in hunter-gatherer times when our immune systems were evolving that might be prompting them to overreact.

The good news is that a better understanding of how the immune system regulates itself – and how organisms such as parasites subvert this system – is spurring the development of new treatments for allergy, and even raising the prospect of a cure.

The advantages of old friends

Some of the strongest evidence for this 'old friends mechanism' comes from studies of people infected with parasitic worms, like hookworm. Severe hookworm infection is a major cause of anaemia and malnutrition in developing countries, and because this adversely affects school attendance and educational attainment, many have initiated deworming programmes. Professor Alison Elliott, who directs the Co-Infections Programme at the Medical Research Council's Uganda unit, has been investigating the impact of deworming on women and their children living on the shores of Lake Victoria. "We've found that if the mother had an infection – particularly hookworm – during pregnancy, then her baby was less likely to develop eczema," Elliott says. What's more, treating women with the deworming drug albendazole during pregnancy significantly increased the chances that their baby would have eczema.[2]

There's supporting evidence from developed countries too. For instance, allergies seem to be more prevalent in city-dwellers compared to people who live in the countryside. One recent study by Professor Ilkka Hanski at the University of Helsinki in Finland found that people living near farms and forests had far more diversity in the types of bacteria living on their skin – including the presence of a genus called *Acinetobacter*, which seem to encourage immune cells to secrete an anti-inflammatory substance called IL-10.[3] Other studies have suggested that exposure to a cowshed during the first six months of life reduces allergy risk – probably for similar reasons.

Evolutionary context

What is happening here? Allergic reactions are a normal immune response to foreign invaders, occurring in an inappropriate context. Their purpose is to expel the foreign particles – be they a bacterium, parasite, or piece of pollen

– from the body, which is why airborne allergies make us sneezy, snotty and weepy, while allergies to food often trigger diarrhoea. In the case of harmful organisms, this makes perfect sense. But most of the proteins that trigger allergic reactions – called allergens – are quite harmless. What's interesting about them though, is how much they have in common with the few proteins that distinguish our own tissue from that of parasitic worms. Increasingly, immunologists suspect that the reason we have allergies could be because the immune system evolved to recognise these proteins and react to them.

"I can't think of any evolutionary advantage to having allergies, but there were advantages to getting rid of worms – or at least keeping their numbers down to a modest level," says Elliott. Because of this, parasites have themselves evolved strategies to dampen down the immune system. For most of human history then, 'old friends' like worms have lived in a kind of equilibrium with the human immune system, to the point where our immune cells expect them to be there. In their absence, these immune cells could become overactive.

Multiple factors

Admittedly, this is unlikely to be the only factor contributing to the rise in allergies in recent decades. A citizen science project called #BritainBreathing from the British Society for Immunology, University of Manchester and Royal Society of Biology is currently gathering symptom data from large sections of the allergic population via a mobile app to try to unpick some of these factors. For instance, in the case of hay fever, pollution has been shown to modify the structure of pollen proteins to make them more allergenic, and the intensification of agriculture has increased the likelihood of vast clouds of allergenic pollen forming in localised areas. We're also being exposed to new pollens, such as ragweed, thanks to the introduction of foreign plant species from abroad.

Even so, increasing the diversity of microbes that we and our children are exposed to may be a simple way to reduce the chances of our immune systems developing a reaction against them in the first place. This doesn't mean not washing our hands – the sorts of microbes that cause food poisoning

or other illnesses don't seem to be the ones that have a protective function. Instead, it means breastfeeding our babies, avoiding unnecessary use of antibiotics that deplete our microbes, and spending more time in the countryside where many of these beneficial bugs are thought to be found.

Imitating parasites

What about people with established allergies? To date, there's little evidence that boosting our exposure to a broad repertoire of microbes can cure allergies. Attempts to curb symptoms of hay fever or asthma by deliberately infecting people with parasites such as hookworm and whipworm, for instance, have so far yielded variable results. Infecting people with live worms is also far from ideal, since they can make some people very ill.

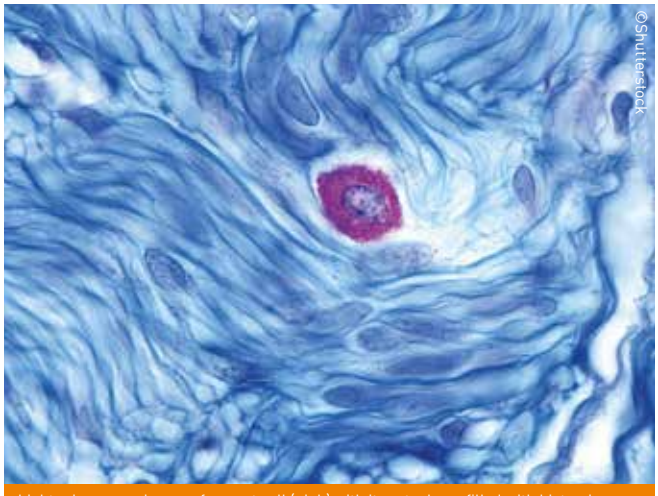
However, work is underway to better understand what parasites are doing to the immune system, in the hope of replicating, or even improving on it. One such group is headed by Professor Rick Maizels at the University of Glasgow. He has been collecting some of the molecular flak that parasites such as the intestinal roundworm, *Heligmosomoides polygyrus*, (which infects mice) release in order to suppress the immune system of their hosts. Of particular interest is a protein called allergic response inhibitor (ARI), which seems to interfere with signalling by an immune system protein called IL33. "IL33 is like an alarm signal given out by the host's cells," says Maizels. "It seems to be the spark that initiates the allergic response." Indeed, when Maizels and his team purified ARI and injected it into mice, it protected them against the development of allergies in the same way that infecting them with whole parasites would. They are now testing whether the same protein can reverse established allergy.

Although *H. polygyrus* is a mouse parasite, and ARI is therefore intended to suppress the immune systems of mice, the gene for IL33 has also been implicated in human allergies – particularly asthma. Pharmaceutical companies are now trying to develop monoclonal antibodies to IL33 for the treatment of asthma. However, "we think what the parasite is doing is a bit cleverer because it is starting upstream of IL33, at the very beginning of the signalling pathway," Maizels says. This could potentially make it more effective: bolting the stable door, rather than trying to catch the horse once it has already bolted. But ARI is less effective in human cells, so its structure may need to be tweaked if it is ever to be used in people.

Re-education of immune cells

Both ARI and existing allergy treatments, such as antihistamines, work by suppressing the immune response. But what about trying to re-educate immune cells so that they no longer mistake these proteins for enemies? That's the idea of desensitisation therapy: currently the closest thing we have to a cure for allergy. Here, immune cells in the lymph nodes are exposed to increasing doses of the problematic allergen – either through regular injections, or drops or tablets under the tongue. Though the precise mechanism remains unclear, this exposure prompts the development of regulatory T cells, which act as a brake on immune responses, ultimately resulting in tolerance of the antigen.

Desensitising immunotherapy is time-consuming and expensive, so for now it's only used for those with severe allergies – although researchers are investigating new ways of delivering allergens, which could make it practical for a larger number of people. It also carries a risk of anaphylaxis, a severe allergic reaction which involves the whole body and can be life-threatening. Finding new ways to stimulate the body's natural mechanisms of reining in wayward immune responses and inducing tolerance is therefore a major objective for many immunologists. Maizels is investigating another parasitic protein that seems to directly mimic a signalling molecule the body uses to stimulate the production of regulatory T cells, which are specialised immune cells that can shut down inflammation. Other researchers, meanwhile, are focusing their efforts on the allergens that initiate allergic responses in the first place.



Light microscope image of a mast cell (pink) with its cytoplasm filled with histamine granules. Outside, the smooth muscle fibers are stained in blue.

HOW ALLERGIES MAKE US SNEEZE AND WHEEZE

in IgE-mediated allergic reactions

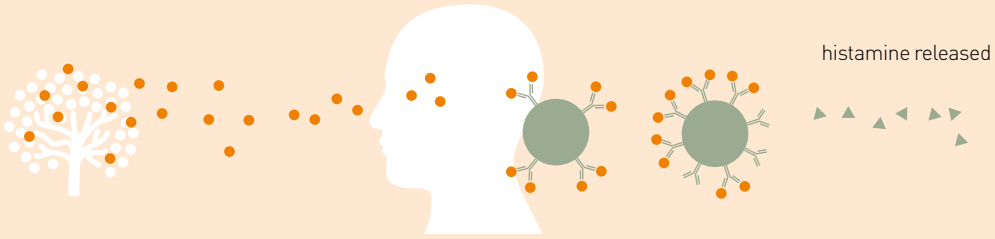
Adapted, with permission, from: *Sense About Science 2015 Making Sense of Allergies*, page 4

Priming exposure to allergen: sensitisation

1. When an allergy-prone person is exposed to an allergen, eg pollen, their body may produce IgE antibodies to that allergen.

2. These antibodies attach to a type of white blood cell called mast cells. This is known as sensitisation.

Next exposure to allergen: allergic reaction



3. The next time the sensitised person is exposed to the same allergen, the mast cells recognise the allergen via the IgE antibodies and release chemicals, such as histamine into the tissues. The chemicals irritate the body and cause allergic symptoms.

A two-step process

The development of allergies is a two-step process. The first stage occurs the first time an allergy-prone person is exposed to a substance such as pollen or pet dander, and involves the presentation of fragments of allergen to helper T cells, which then stimulate B cells to produce IgE antibodies against it. These circulate until they encounter another type of immune cell called a mast cell. These loiter in barrier sites of the body, such as the skin and lungs, and then grab onto the antibodies that pass by and keep hold of them. The next time you encounter that allergen – even if it's not for months or years later – those primed mast cells will bind to it and become activated.

"Mast cells are packed with chemical weapons like histamine, and once they're activated they immediately start spewing out these chemicals," says Dr Sheena Cruickshank, who studies the initiation of immune responses at the University of Manchester. These chemical payloads help to recruit more immune cells to the site, make the blood vessels leaky (so these immune cells can get into the tissue), and help to produce mucus; the result is swelling, the secretion of fluid to try and flush the invader away, and the triggering of explosive responses like sneezing. "Mast cells are very good at protecting against infections, so they are important cells, but unfortunately in allergy they are bad," says Cruickshank.

Context is everything

As well as helping B cells to produce antibodies, T cells are also involved in influencing the behaviour of mast cells and other cells involved in mounting an immune response. But the context in which the T cell sees the allergen is extremely important: if IgE antibodies on mast cells have bound to the allergen and triggered inflammation, T cells will amplify that response. If the T cell encounters the allergen in the absence of inflammation, it will encourage the development of regulatory T cells, ultimately resulting in tolerance. "We think T cells are what really create and sustain the allergic response," says Mark Larché at McMaster University in Ontario, Canada.

Crucially, T cells respond to fragments of allergens, whereas mast cells need to see the whole thing in order to become activated. So Larché is searching for short stretches of amino acids within allergens that have the properties enabling T cells to see them. "The idea is that we get rid of all the elements that trigger allergic responses, whilst retaining the important stuff that can be used to target T cells," he says.

So far, his team has identified a number of short peptide sequences from cat dander, grass pollen and house dust mite, which are currently in phase 2 and 3 clinical trials. In one such trial, volunteers who were injected with the cat dander peptides four times over the course of three months, reported a 3.9-point improvement in their allergic symptoms. This compares with an average 1.1-point improvement for traditional immunotherapy, which involves regular injections over several years. They also reported fewer adverse events with peptide therapy compared to traditional immunotherapy.[4] Further studies are ongoing.

While it's too early to say whether such approaches will truly yield a cure for allergy, these insights into the crosstalk between immune cells and the delicate balance it maintains between suppression and inflammation are at least grounds for optimism. At the moment, you'd be hard pushed to find a classroom without at least one allergy sufferer in it; perhaps in another 50 years this debilitating condition will once again be viewed as an anomaly rather than the norm.

1. House of Lords 2007 The extent and burden of allergy in the United Kingdom. Chapter 4 in: Select Committee on Science and Technology – Sixth Report
2. Mpairwe *et al.* 2011 *Pediatric Allergy and Immunology* **22** 305–312
3. Hanski *et al.* 2012 *Proceedings of the National Academy of Sciences* **109** 8334–8339
4. Patel *et al.* 2013 *Journal of Allergy and Clinical Immunology* **131** 103–109

The enemy within?

New perspectives on autoimmune disease

New knowledge generated by original basic science takes an average of 17 years to reach the clinic. That's the conclusion reached by a number of authors who have sought to quantify what some have called the translational research time lag.

That may be a depressing thought for PhD students motivated by a desire to rapidly improve the lot of patients. For those working in autoimmunity (the study of how the body's immune system can attack its own cells to cause disease), however, the figure underlines the feeling in the field that significant clinical returns on the investment in basic research going back 30 years are imminent.

The dawn of a golden age?

Our understanding of the immune system and autoimmune conditions, such as type 1 diabetes, rheumatoid arthritis and inflammatory bowel disease, has grown rapidly in the last 30 years or so. Of particular importance has been research that has revealed the fundamental part played by T cells in a range of essential roles to regulate immune responses.

Over this time better treatments have been developed, yet some are disappointed that lasting cures have not been found. "There's been a lot of progress, but the really important progress of getting patients close to cures is still to come," says Professor Sir Marc Feldmann, who in 2014 was awarded the Canada Gairdner International Award, which in many cases has been a precursor to a Nobel Prize, for the discovery of anti-TNF therapy, a treatment now used for a range of inflammatory autoimmune conditions such as rheumatoid arthritis. Many in the field believe we are at the dawn of a golden age which will see major benefits for patients in the form of both treatments and cures.

Anti-inflammatory breakthroughs

While science is a highly collaborative and international endeavour, few would dispute that UK-based immunologists have made major contributions to this field, including for example the elucidation of the central roles of T cells. In the early 1980s, research from various groups showed that human leukocyte antigen (HLA) genes, which encode major histocompatibility complex (MHC) cell surface proteins, are upregulated in autoimmune disease-affected tissue. Feldmann, who was studying the role of HLA in triggering T cell activity, hypothesised in a 1983 paper that cell signalling molecules called cytokines, which upregulate MHC, were key to understanding the triggering of autoimmunity.

Together with Professor Sir Ravinder Maini, then at the Kennedy Institute of Rheumatology in Oxford, Feldmann began to look at the cytokines expressed in joint tissue from patients with rheumatoid arthritis. "The dilemma was there were about a dozen proinflammatory cytokines present, all at levels capable of upregulating inflammation," says Feldmann. "Most researchers in the field concluded they were not good targets for therapy because if you blocked one cytokine, the others that were present would still drive the biology so you would be wasting your time."

Feldmann and Maini disagreed with this view, and went on to show that excessive production of one cytokine called tumour necrosis factor alpha (TNF-α) causes the inflammation that occurs in inflammatory joint disease by demonstrating that blocking it prevents the production of the other proinflammatory cytokines in their model of human disease tissue in culture. They further went on to lead clinical trials which demonstrated impressive anti-inflammatory effects. This eventually led to the approval of the first widely used monoclonal antibodies, the anti-TNF-α drugs, in 1998. These have gone on to become the treatments of choice to stop inflammation in rheumatoid arthritis and other autoimmune conditions including ulcerative colitis, psoriasis, Crohn's disease and ankylosing spondylitis.

Regulatory T cells on the map

The 1990s saw important advances in the discovery and description of cells that suppress immune reactions. Work by both Professor Shimon Sakaguchi, of Osaka University and Professor Fiona Powrie in Oxford helped identify the roles of regulatory T cells in self-tolerance, and how their malfunction could cause autoimmune diseases. In 1990, Powrie showed that injecting one set of T cells into rats could cause inflammatory disease; however if regulatory T cells were injected at the same time the rats were protected. "Between them, Powrie and Sakaguchi put regulatory T cells and immune regulation on the map," says Lucy Walker, Professor of Immune Regulation at University College London. "That was really important in advancing our understanding of autoimmunity, and what prevents autoimmunity normally."

Around this time Professor Herman Waldmann, at the University of Oxford, was developing his idea of infectious tolerance. In a key paper published in the journal *Science*, he demonstrated that courses of CD4 antibodies could stimulate long-term immune system tolerance to foreign proteins, and that this tolerance could be transferred from one animal to another by transplanting the right immune cells. Waldmann went on to demonstrate that the regulatory T cells Powrie had described were required for infectious tolerance.

Helper T cells, also known as CD4 cells, play a crucial role in protecting the body from pathogens by triggering the release of cytokines that suppress or moderate immune responses. It was initially thought they could differentiate into just two subsets. These were type 1 (Th1) to fight viruses and other intracellular pathogens, eliminate cancer cells and stimulate delayed type hypersensitivity skin reactions, and type 2 (Th2) to stimulate antibody production to combat extracellular organisms. In fact, recent research has shown they can turn into other types as well. Professor Gitta Stockinger, now at the Francis Crick Institute in London, for example, has been instrumental in the discovery of Th17 cells that produce the proinflammatory cytokine interleukin-17, which in turn has been found to play an important role in autoimmunity.

A finely balanced system

The growth in our knowledge about the different types of T cells has greatly improved our understanding of the causes of autoimmune diseases. "It has made us think about autoimmunity in terms of both the cells that induce disease and the cells that protect from disease," says Walker. "In thinking about therapies, we traditionally thought about trying to block dangerous cells to stop them working. More recently there has been much more emphasis on trying to boost the protective cells in the regulatory arm of the immune system."

Work by Stockinger on Th17 cells and others on the wide variety of ways that T cells can become activated has stimulated many in the field to investigate the types of T cell activation involved in different autoimmune diseases. In work published last year, for example, Walker's group identified a central role of follicular helper T (Tfh) cells, which help B cells in the development of humoral immunity, in type 1 diabetes. Humoral immunity is the element of the immune system associated with antibodies found in extracellular fluids. Walker found that memory T cells from type 1 diabetes patients exhibited enrichment of Tfh cells and greater secretion of the soluble protein interleukin-21. "This may provide a way to track levels of Tfh cells in patients following therapy to see if treatment is working, and inspire ideas about different pathways to target to combat the disease," says Walker.

Following on from the identification of regulatory T cells and their roles, researchers have sought to identify the mechanisms by which they act to suppress immune responses. A key way they do this is through the CTLA-4 protein. In collaboration with the laboratory of Professor David Sansom, Walker's group identified a novel molecular mechanism for CTLA4 function and, together with colleagues at the Royal Free Hospital in London, reported in 2014 that patients with CTLA4 gene mutations exhibit a wide variety of autoimmune symptoms. Samples of regulatory T cells taken from their blood cannot perform their normal immune regulation function. A soluble version of CTLA-4 called abatacept is used to treat some rheumatoid arthritis patients and other autoimmune diseases, and may prove a useful therapy in CTLA4 deficiency.

Helper T cells lend a hand

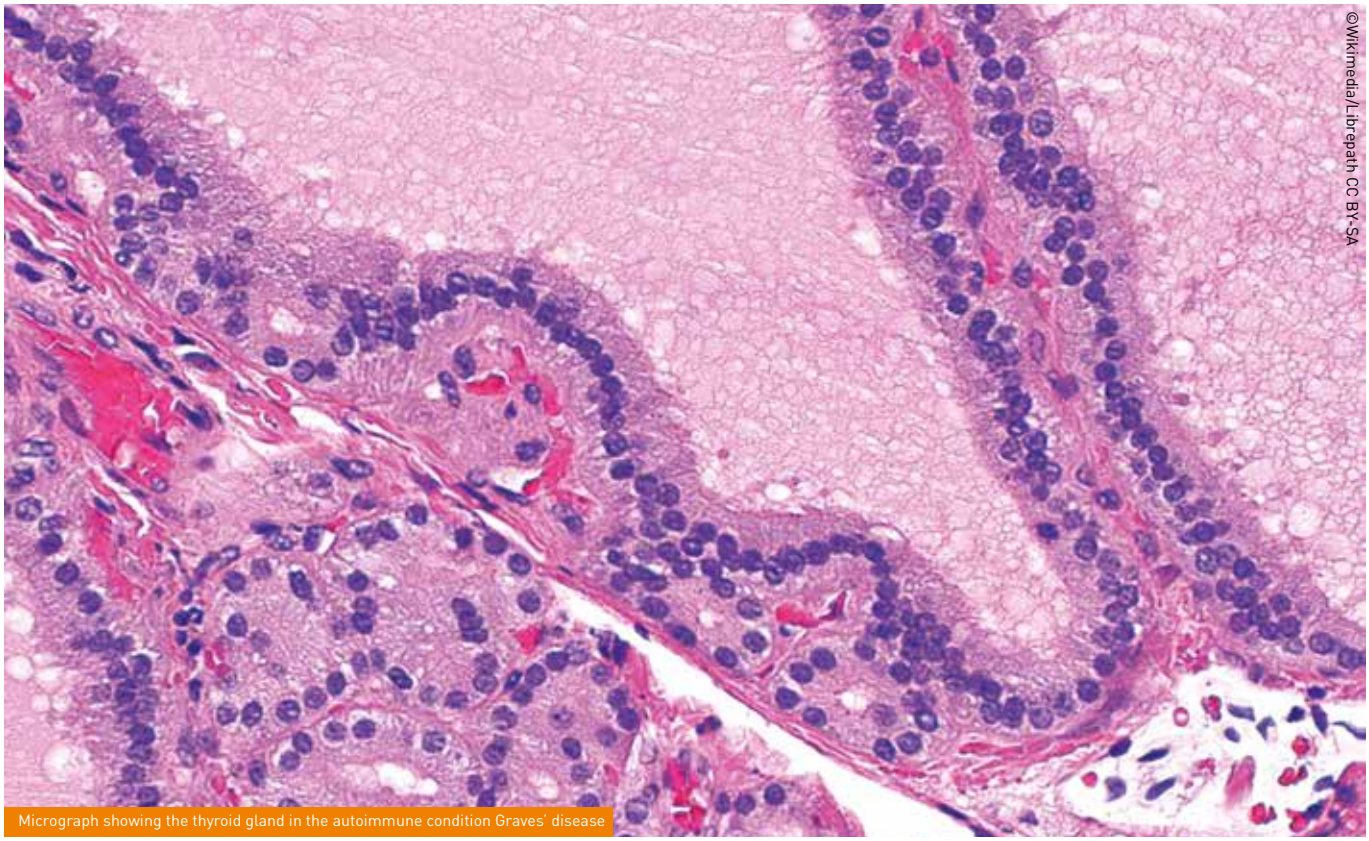
The central role of helper T cells in autoimmune disease was revealed by research during the 1980s and 1990s. Professor David Wraith, now at the University of Birmingham, believed that finding ways to desensitise helper T cells could offer new therapies. He was intrigued by the idea of adopting the 'antigen-specific immunotherapy' approach that has been used against allergies since the time of John Freeman and Leonard Noon, of St Mary's Hospital, London, who published a trial on it in *The Lancet* in 1911.

Others had tried this approach in autoimmune diseases before using whole or intact antigens, however the mechanisms involved were not fully understood. Such efforts proved largely ineffective and some triggered harmful autoimmune responses. An important clue to a way forward lay in research showing associations between autoimmune diseases and genes that encode protein receptors for small fragments of antigens called peptides. Further work showed it was these rather than the whole antigen that helper T cells were responding to.

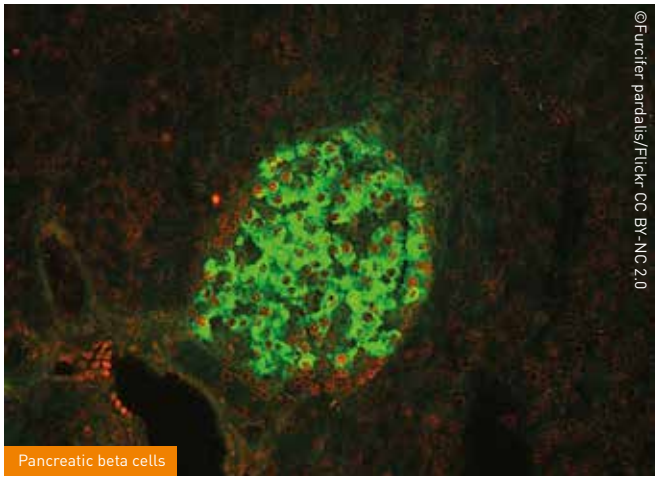
Wraith set about demonstrating it was possible to suppress immune reactions in autoimmunity using synthetic versions of these peptides. Some warned this could make things worse. "That's not how it turned out," says Wraith. "What we came to realise was we weren't just switching these cells off. We were turning them from potentially aggressive cells that could promote autoimmune disease into ones that could police the immune system and protect against autoimmune disease."

Clinical trials

Getting funding to carry out clinical trials of peptide immunotherapy proved difficult. Wraith set up a company called Apitope in 2002. A phase I trial published in 2015 found injections of ATX-MS-1467, a treatment based on this approach, to be safe and well-tolerated in six patients with secondary progressive multiple sclerosis. Merck Serono has licensed the treatment and has provided backing for two more trials. Wraith says that initial



Micrograph showing the thyroid gland in the autoimmune condition Graves' disease



Pancreatic beta cells

Fingers with rheumatoid arthritis

MRI scans have shown the approach can significantly reduce the inflammation and scarring to the protective myelin sheaths that surround nerves that are characteristic of multiple sclerosis.

"Current therapies for autoimmune diseases tend to rely on immunosuppressive drugs that have to be given long-term and make patients susceptible to infections and cancers," says Wraith. "We want to get away from non-specific immune suppression and help the immune system correct itself. What I want to see in the last part of my working career is this approach being rolled out into as many diseases as possible."

Apitope is working on therapeutic peptides for the thyroid condition Graves' disease, uveitis (which can cause vision loss) and the rare chronic bleeding disorder Factor VIII intolerance. Professor Mark Peakman, at King's College London, completed a trial of a peptide-based treatment for type 1 diabetes last year, and began a trial of a more powerful version called MultiPepT1De in 24 patients at Guy's Hospital, London, in March. A phase III trial of Lupuzor, a potential therapy for the autoimmune condition lupus, was launched by the UK-based pharmaceutical company ImmuPharma last year.

> *"While science is a highly collaborative and international endeavour, few would dispute that UK-based immunologists have made major contributions to this field"*

Boosting regulatory processes

Various groups around the world are pursuing another approach widely seen as promising – boosting regulatory T cell activity to counter autoimmunity, either by growing them in the lab or finding other ways to increase their activity. The soluble protein interleukin-2 (IL-2) is known to increase the number of regulatory T cells naturally and there is considerable interest in using this as a therapy.

Dr Frank Waldron-Lynch, Professor John Todd and Professor Linda Wicker of the University of Cambridge have completed a trial of synthetic IL-2 to determine the optimal dose in patients with type 1 diabetes and are carrying out another to find out the optimal treatment frequency, in preparation for a phase II trial.

Professor Giovanna Lombardi, of King's College London, adopted the other approach of taking regulatory T cells from 13 patients with Crohn's disease and growing them to increase their number in the lab using IL-2. In research published in 2014, her group showed that, *in vitro* at least, the regulatory T cells they had grown could modulate immune responses seen in the inflamed tissue of Crohn's patients.

Millions of rheumatoid arthritis patients already benefit from anti-TNF drugs. There are other treatments for autoimmune conditions that work by dampening immune system responses based on targeting the interleukin 6 receptor (IL6R) and B-lymphocyte antigen CD20. These are known as 'biologics' – genetically-engineered protein therapies derived from human proteins. The recent success of immune checkpoint inhibitor-based cancer immunotherapy in boosting the immune system's response to certain forms of cancer leads many to believe there is potential for major clinical improvements for those with autoimmune conditions based on doing the reverse, especially for treatments that target T cells.

"There's a real sense that immunology is coming of age," says Walker. "Biologics have been seen as highly successful in augmenting the immune system to fight cancer. The hope is that in autoimmunity we can develop further treatments that do the opposite to suppress the immune system to the real benefit of patients. It's a hugely exciting time."

The last frontier?

Lifting the lid on the blood–brain divide

While vaccines and infectious diseases provide major challenges for immunologists, there are other aspects of immunology that are no easier to crack. For many years the brain was considered a fortress cut-off from the rest of the body. A hard bony skull protected it from outside threats, while the tight cellular junctions of the blood–brain barrier shielded it from inner ones – including the immune system. There were good reasons for this assumption: immune activation is associated with inflammation and swelling – processes there's little space for within the rigid confines of the skull – while cell death and the regeneration it unleashes would surely result in the disruption of learned actions and memories.

These assumptions were backed up by physical evidence, too. With the exception of microglia – immune-derived support cells that populate the brain – there was little sign of any immune cell activity in the way of peripheral white blood cells such as T and B cells in the central nervous system. Additionally, early experiments that involved transplanting foreign tissue into the brain failed to prompt the usual immune rejection. "Because we couldn't really see any evidence of any white blood cells in there, the possibility of having an immune response in the brain was thought to be remote," says Clive Holmes, Professor of Biological Psychiatry at the University of Southampton. The discovery of T and B cells in the brains of people with multiple sclerosis only confirmed suspicions that when the immune and nervous systems got together, bad things happened.

Infiltrating the brain

The past 20 years has seen a dramatic re-evaluation of this model. Not only can white cells from the blood infiltrate healthy brain tissue, their presence might be a necessary means of keeping out and stopping foreign invaders like viruses from causing damage. Immune cells also gather on the meninges – a fibrous membrane that surrounds the brain and central nervous system – and communicate with a whole network of microglia and astrocytes in deeper brain structures.

Our understanding of the function of these immune-derived cells has also been turned on its head: once considered passive support cells, we now know that microglia are crucial in shaping the neuronal network by trimming away weak connections between neurons. Both microglia and astrocytes release signalling molecules of their own to influence brain function as well as producing growth factors that aid neuronal growth during development and also repair neuron and myelin damage in diseases such as multiple sclerosis.

However, as well as being an essential component of healthy brain function, this brain-immune relationship could have a dark side. From Alzheimer's to Parkinson's disease, schizophrenia and depression, diseases that were once considered to be purely neurological are increasingly being linked to a dysfunctional immune system – which could in turn open up new opportunities for treating them.

Sickness behaviour

Some of the first clues that the immune system might be influencing the brain came from studies of sickness behaviour, a coordinated set of symptoms including lethargy, depression and loss of appetite that will be familiar to anyone who has been ill. "When people get a peripheral infection, they feel psychologically impaired by it," says Holmes. "They might have mood changes and loss of concentration, which implies that there is something going on in the brain."

These same symptoms can also be triggered in experimental situations following an injection of lipopolysaccharide (LPS) – a major component of some bacterial outer cell membranes – into the bloodstream, suggesting it's not simply being ill that causes this behaviour. Indeed, experiments have revealed that an injection of LPS prompts immune cells to release inflammatory cytokines that both stimulate the vagus nerve (a major communication channel into the brain) and immune-like cells living on the brain's edges, which in turn activate microglia deeper in the brain.

All of this makes sense from an evolutionary perspective: if you feel tired and anti-social when you're fighting off an infection, you're less likely to go out and spread that infection to other people, or pick up more infections when your immune system is already activated.

> *"There's a growing sense that T cells need to go into the brain – that they have this surveillance role in health – and they almost certainly go in during infection"*

Immune functions within the healthy brain

The immune system may also play a role in the healthy brain. Experiments by Professor Jonathan Kipnis at the University of Virginia and his colleagues have revealed that mice engineered to lack CD4+ T cells perform poorly on learning and memory tasks, but improve if they are injected with T cells taken from healthy mice. Further research has revealed that learning a new task seems to prompt a mild stress response in the brain which causes T cells to rally to the meninges, where they release signalling molecules that prompt astrocytes to release a protein called brain-derived neurotrophic factor that enhances learning. "Even though the immune cells are sitting at the edges of the brain, they are still important for its function," says Kipnis.

However, T cells may be able to penetrate into the brain's deeper layers as well. For a long time this was only thought to occur in neurological diseases such as multiple sclerosis, when aggressive immune T cells primed to attack the myelin coating that speeds up transmission of nerve signals broke through the blood–brain barrier. However, advances in microscopy and the ability to fluorescently tag immune cells have recently revealed that the passage of these T cells into the brain is an active process that involves the cooperation of healthy cells living in the meninges. "There's a growing sense that T cells need to go into the brain – that they have this surveillance role in health – and they almost certainly go in during infection," says Professor Sandra Amor, Head of Multiple Sclerosis Research at VU University Medical Center in Amsterdam. Indeed, T cells may play an important role in regulating immune responses within the brain and ensuring they don't get out of control.

Lymphatic vessels have also recently been discovered in the meninges that shuttle molecules and immune cells from the cerebrospinal fluid surrounding the brain and spinal cord to a group of lymph nodes buried deep in the neck. "We think immune cells in these lymph nodes will see these molecules, become activated and then go back into the brain and perform their effect," says Kipnis, who led the team that discovered them. In most cases, the immune cells will travel to the meninges and release signals that indirectly influence brain function via the microglia. But in extreme circumstances, immune cells may cross the blood–brain barrier and influence the brain more directly
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New approaches for multiple sclerosis

Such discoveries are prompting a radical rethink of the role the immune system plays in the healthy brain. "Even twenty years ago, if you told someone that the central nervous system recruits immune cells into its tissues for its benefit, they would think you were crazy. Today, the question is not: are they beneficial or not, but what exactly are they doing, and how can we augment the beneficial response?" says Kipnis.

So what could this new understanding of the brain's immune system mean for patients? It's early days, but these insights are already translating into new therapies for people with multiple sclerosis (MS), a neurological condition characterised by damage to the protective myelin coating that surrounds nerve fibres. An early example is natalizumab (Tysabri) – a monoclonal antibody-based drug which targets a molecule on the lining of blood vessels that T cells bind to in order to gain entry to the brain. This approach is very effective in blocking T cells going into the brain and thus very effective in early MS. The trouble is that taking this drug also raises the risk of developing another rare, but severe, brain disease called progressive multifocal leukoencephalopathy, which is triggered by the John Cunningham virus – further evidence that T cells play a role in healthy brain function. "If you're blocking all the T cells from going into the brain, you're also going to block the good ones that control such infections" Amor points out.

Another strategy might be to target microglia instead. Whereas MS has long been considered a disease of dysfunctional T cells, Amor believes they might be a secondary consequence of something happening to the oligodendrocyte cells that produce myelin, and the microglia they communicate with. Specifically, the microglia seem to become activated in response to a kind of stress signal put out by the oligodendrocytes – though what triggers this signal is unknown.

"Generally T cells don't go into the brain in large numbers unless they're called in for some reason – maybe because the microglia can't control the situation and they need back up by the peripheral immune cells," says Amor. "In most cases the microglia and astrocytes can control so-called danger situations but are not armed with munitions to fight major battles. In these cases, signals are sent to the T and B cells to enter the central nervous system." Once the T cells reach the site of the problem, Amor believes the T cells start attacking the oligodendrocytes, resulting in the loss of myelin. However, as the disease progresses there is accumulating evidence that microglia, rather than T cells, play a role in the neurodegeneration that occurs. Developing drugs that directly target microglia might therefore be an alternative therapeutic option. One such approach that has been shown to modulate microglia in experiments is a heat shock protein called HSPB5. It has been used in clinical trials in early MS, but has not yet been tested in late disease.

Alzheimer's implications

Dysfunctional microglia are also the focus of new strategies to treat and prevent other brain conditions. Take degenerative diseases like Alzheimer's. Until recently, there was little evidence that the immune system played any role in this disease; most research had instead focused on the amyloid plaques that are its hallmark. Yet there's a growing suspicion that, at the very least, infection or inflammation outside the brain might be part of the problem. It might even be the initial trigger for amyloid production.

One of the first clues that the immune system could be involved in Alzheimer's disease came from observations of mice predisposed to develop neurodegenerative disease. When Professor Hugh Perry at the University of Southampton injected these mice with LPS to mimic a bacterial infection outside the brain, their microglial cells became more activated, their neurons started to die, and their performance on cognitive tasks began to suffer. Intrigued, Perry called Holmes, and asked whether his Alzheimer's patients similarly deteriorated if they got an infection. "Of course they do," Holmes replied. But when he turned to the published literature, he could find little to back up his assertion and so he started researching it himself.

Cognitive declines

Since then, he and Perry have discovered that it's not just infection, but chronic inflammation caused by other diseases such as rheumatoid arthritis, atherosclerosis, and even gum disease, that can hasten the cognitive decline of people with Alzheimer's. "Infections, such as urinary tract infections, roughly double the rate of decline, while chronic low grade inflammation increases it about four-fold," says Holmes.

He suspects that the presence of amyloid somehow primes microglia, putting them into a state of high alert. If they then encounter inflammatory signals coming from elsewhere in the body they overreact, and ultimately start killing brain cells. "We think these very low grade infections – things that you or I wouldn't necessarily even be aware of – are enough to cause major damage in people with Alzheimer's," Holmes says.

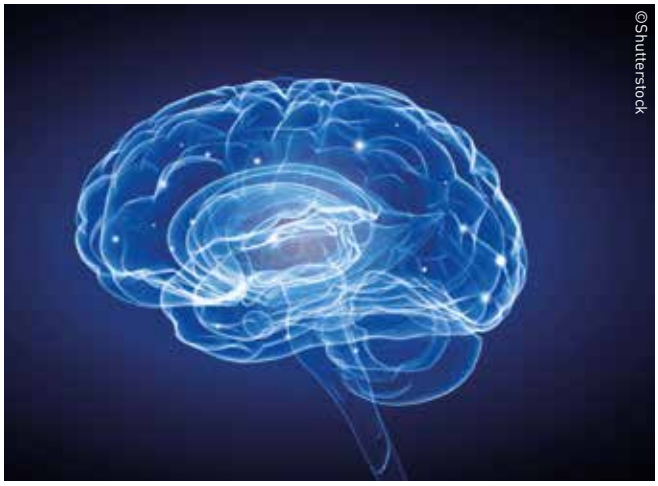
Inflammation could even be what triggers the production of amyloid in the first place. Alzheimer's is a highly heritable disease, and of the genes that have been linked to the most common form of Alzheimer's so far, around half are involved in inflammatory processes. Animal studies have demonstrated that infections elsewhere in the body can trigger amyloid production in the brain, while studies in cell culture have suggested that amyloid has antimicrobial properties. "Possibly it's a protective mechanism against bacteria entering the brain," says Holmes.

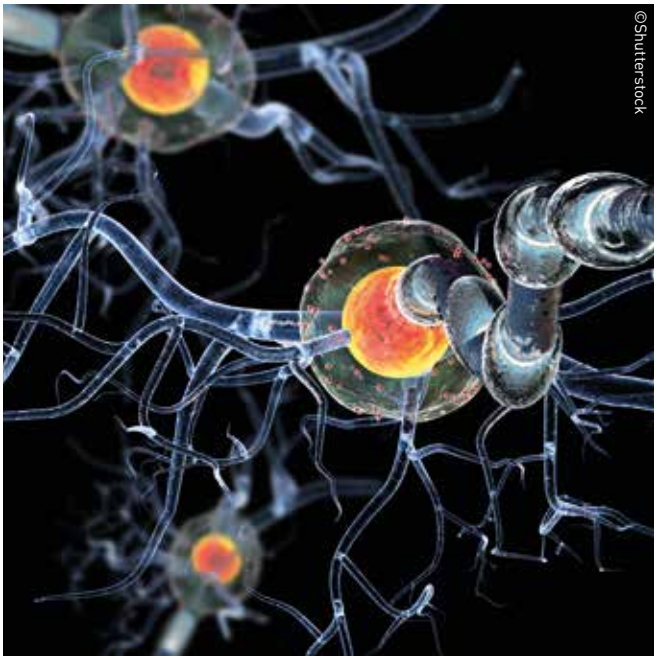
New leads to novel treatments

Such discoveries raise the prospect of using anti-inflammatory drugs to treat the disease; indeed, epidemiological studies have suggested that people who take non-steroidal anti-inflammatory drugs (NSAIDs) are at reduced risk of developing Alzheimer's. However, trials that have involved giving NSAIDs prospectively have produced mixed results. Possibly this is because the drugs they used aren't specific enough. One cytokine that has consistently been associated with cell damage in the brain is TNF-α, and "a lot of non-steroidal drugs don't hit TNF-α at all," says Holmes. In a small pilot study of 41 patients, he and his colleagues gave patients either the TNF-α blocker etanercept or a placebo for six months. Those on etanercept saw no progression of their disease while those on the placebo drug deteriorated.

TNF-α blockers are also being tested in people with depression. Here too, the link between systemic inflammation and psychological symptoms has been growing for some time, and activated microglia seem to be involved in at least a subset of cases. People with multiple sclerosis, diabetes and rheumatoid arthritis all have higher than average baseline levels of inflammation, and are at greater risk of depression. "This risk seems to be separate from the disease itself," says Amor.

> *"Not only can white cells infiltrate healthy brain tissue, their presence might be a means of keeping out and stopping foreign invaders like viruses from causing damage"*





In one recent trial, 60 people with treatment-resistant depression were either given the TNF-α blocker infliximab, or a placebo drug over 12 weeks. Although at first glance there was little difference in the outcomes of the two groups, when the researchers focused in on volunteers who had started out with high levels of inflammation, those in the infliximab group showed an improvement in their symptoms.

A new age of understanding

Although we've come a long way in our appreciation of the role the immune system plays in the brain, there's still plenty we don't understand. Why, for instance, does inflammation result in depression in one individual, and dementia in another? "Clearly the triggers of the brain's immune system are different in these diseases," says Amor. And how could ageing and the changes in immune status it brings affect the brain? "I think we're just at the very tip of the iceberg in terms of our understanding," says Kipnis.

But one thing is now certain: the brain is anything but isolated from the rest of the body. By studying the immune system, we're likely to learn far more about what makes us tick than by considering the brain alone.

Immunotherapy: the next era of cancer treatment

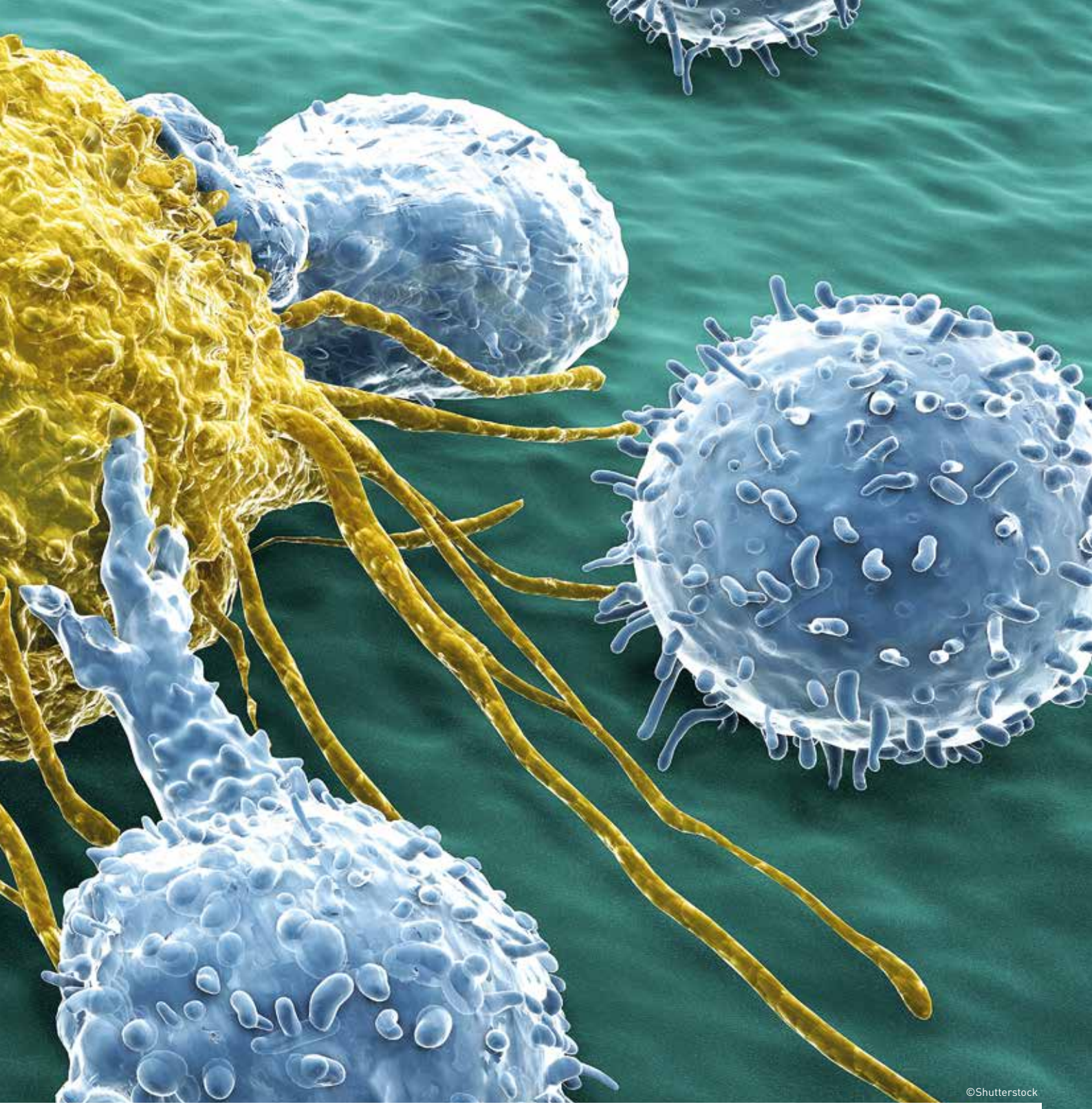
The tumour on the man's tonsil was the size of an egg; it bulged out from his neck and obscured so much of his throat that he could barely swallow. Emaciated and weak, no-one held out much hope that he would survive. Deliberately injecting him with bacteria that would cause his skin to blister and his temperature to soar might therefore have sounded like a cruel form of torture. But William Coley, the surgeon brandishing the syringe, hoped it would prove his salvation. Indeed, in the months following the injection in May 1891, the patient's tumour began to break down, and by October it was gone.[1] The man lived a further eight years before the cancer relapsed and ultimately killed him.

This was some of the first evidence that stimulating the immune system – in this case by triggering an infection – might cause cancers to regress. In the years that followed, Coley refined his technique and claimed to cure many more patients, although others struggled to replicate his results and following his death in 1936, Coley's toxins were gradually forgotten.

Today though, the idea of harnessing the immune system to fight cancer is firmly back on the agenda. A string of successful trials involving immune-based drugs called checkpoint blockers or inhibitors – not to mention the recovery of the former US president Jimmy Carter from melanoma – has seen pharmaceutical company investment in the field soar. Immunotherapy is also at the heart of the recently launched 'Cancer MoonShot' initiative in the USA, the goal of which is to find a vaccine-based cure for cancer by 2020.

A shape-shifting enemy

Is such excitement justified? History tells us that cancer is a shape-shifting enemy, and molecular therapies – previously hyped as a silver bullet for cancer – have been less successful than many had initially hoped. Yet there are several reasons to think immune-based therapies might do better. The first is immunological memory, which means that once cells of the immune system are engaged in fighting a tumour, they should continue to do so – even if the cancer disappears and then returns at a later date. The immune system is also capable of adapting to changes in its enemies through such phenomena as epitope spreading, in which immune cells diversify to attack multiple targets, as well as the one they started with. "This means even if tumour cells evolve and sub-clones emerge, it may be possible for the immune response

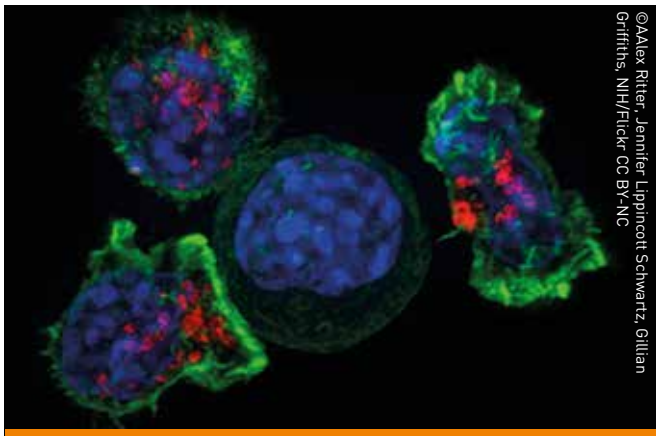


to continue recognising them," says Peter Johnson, Professor of Medical Oncology at the University of Southampton and Chief Clinician for Cancer Research UK. "The emergence of resistance is a problem for molecular therapies."

Modern interest in harnessing the immune system has been building since the 1980s when experiments in mice revealed that it was possible to immunise them against developing a particular type of tumour, if the cancer cells were first mutagenised by exposing them to radiation or chemicals.[2] Before this, many scientists had assumed that cancer cells were too similar to our own cells for the immune system to recognise them. One of the main issues seems to be transforming this initial recognition of the cancer cells into a full-blown immune attack on them.

The rise of monoclonal antibodies

A major turning point was the development of monoclonal antibodies, which can be raised against a protein of interest and then manufactured in large amounts. One of the first monoclonal antibodies to become available was rituximab, which binds to a molecule called CD20 on the surface of immune cells called B cells and destroys them. Since dysfunctional B cells are the cause of many lymphomas and leukaemias, it's an excellent way of removing them from the body. "From the moment rituximab was introduced as a widespread treatment for lymphoma, we've seen a fall in mortality rates," says Johnson. Other monoclonal antibodies to treat a variety of cancers soon followed, including trastuzumab (Herceptin) and bevacizumab (Avastin). However, the really big shift in the



Superresolution image of a group of killer T cells (green and red) surrounding a cancer cell (blue, centre).

field – and the one currently generating all the excitement – was the use of antibodies to target, not the tumour cells themselves, but the immune system's own control processes.

Taking the brakes off

Because of its destructive power, the immune system has evolved a whole repertoire of regulatory processes to ensure its full might is only unleashed in the appropriate circumstances. "It is a bit like driving a car with one foot on the accelerator and one on the brake at the same time; there are all these checks and balances, which mean the immune response can increase or decrease in a controlled manner," says Dr John Maher, Clinical Senior Lecturer in Immunology at King's College London.

Many of these interactions take the form of molecular handshakes between proteins on the surfaces of different immune cells – or even on the tumour itself. For instance, T cells possess a protein called PD-1 on their surface, which interacts with a different protein that some tumour cells produce in abundance called PD-L1. When this handshake occurs, a brake is applied to T cells, encouraging them to hold fire, rather than attack the tumour.

Pembrolizumab – the drug that Jimmy Carter attributes his recovery from melanoma to – is referred to as a checkpoint blocker. It binds to and blocks PD-1, effectively taking the brakes off T cells and enabling them to mount an effective anti-cancer response.

Lagging only slightly behind the checkpoint blockers in terms of development are antibodies designed to switch on specific immune responses, such those targeting CD40 on antigen-presenting cells (APCs). APCs are responsible for showing T cells the particular proteins (called antigens) that they should react against, thereby kicking off immune responses; antibodies that bind to CD40 seem to activate APCs.

However, such antibody-based therapies are not a panacea. Take checkpoint blockers: they seem to be most effective in cancers that have a high mutational load (i.e. lots of changes to the DNA) – things like skin or lung cancer that often arise following damage by UV light or carcinogens – but even then, only around 20–30% of people respond to them. "The sad reality is that checkpoint blockers do not work for the majority of patients, and so there is still a huge unmet need for additional approaches," says Maher.

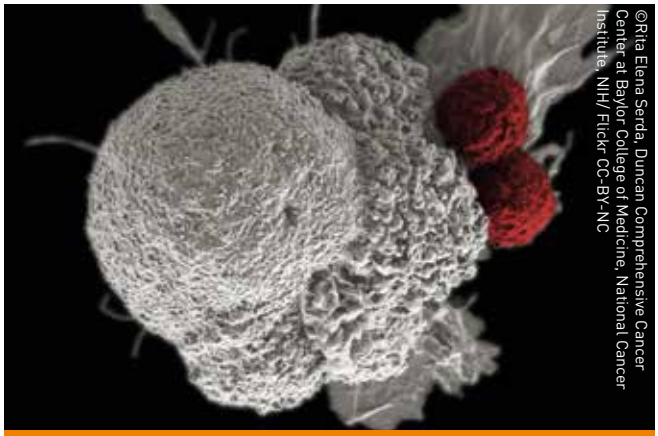
A combined response

One such approach involves a fundamental redesign of T cells. Once set in motion, T cells are highly effective cancer killers, but tumours have evolved many ways of hiding from them. Antibodies, on the other hand, are extremely good at locating tumours, but not so good at destroying them. Chimeric antigen receptor (CAR) T cells are hybrids of the two: T cells that researchers have extracted from a patient's blood and issued with the genetic instructions to make cancer-hunting antibodies as well as their usual T cell receptor. Some of them also contain additional signalling elements, which amplify the T cell's response once it binds to its target. These CAR T cells are then injected back into the patient and left to do their work.

Choosing the right molecular target is crucial: get it wrong, and the T cells will start to attack healthy tissue. But finding targets that are only expressed on cancer cells is tough, because cancer cells derive from our own tissue. The biggest success story to date involves CAR T cells engineered to recognise a molecule called CD19, which is expressed on both malignant and healthy B cells. A pilot study of three patients with advanced chronic lymphoblastic leukaemia who were injected with these cells demonstrated that they could indeed hunt down and destroy their targets – and generate a population of memory cells that could potentially destroy cancerous cells if they returned.[3] However, there's a catch: they also destroy healthy B cells. That's not such a problem, because we can replicate their main function by giving patients antibody replacement therapy; however, this wouldn't be so easy with tumours that affect other tissues, such as the liver or brain.

Target limitations

"The Holy Grail for CAR T cells is the identification of target molecules which are expressed on a sizeable proportion of tumours or leukaemias, and can't be detected on the surface of healthy cells," says Maher. "But that's a very, very short list." Another potential hurdle faced by researchers developing CAR T cells is the possibility of cancer cells mutating, so that they no longer express the T cell's target. In an attempt to combat this, Maher's group is developing T cells that will recognise an entire group of proteins called the ErbB family, which is implicated in a number of different cancers. "It is a collection of eight different targets, which makes it difficult for the tumour just to take out one of them," Maher says. ErbB proteins are also produced by healthy cells, but Maher is getting around



Pseudo-coloured scanning electron micrograph of an oral squamous cancer cell (white) being attacked by two cytotoxic T cells (red).

this by injecting the T cells directly into the tumour rather than into the blood. His team is currently conducting a safety study in terminally ill patients with head and neck cancer. There's no doubt that CAR T cells are an extraordinarily clever means of manipulating the immune system, but whether they will ever become a mainstream cancer therapy is less certain. "We are seeing tremendous efficacy in acute lymphoblastic leukaemia, which has caused a great deal of excitement," says Maher. "However, this is a very toxic treatment."

Preventative measures

Engineering the immune cells of individual patients is also extremely labour intensive, and therefore costly. Far better would be to find a way of preventing cancers from developing in the first place. For one thing, it is easier to mount an immune response against a tumour when it is in its infancy, before it has grown a support tissue called the stroma, which largely protects it from the immune system. "Solid tumours put up a huge wall around themselves as they grow," says Maher.

One such preventative cancer vaccine already exists. The HPV vaccine targets proteins made by the human papilloma virus – the main cause of cervical cancer worldwide. Other viruses including Epstein-Barr and hepatitis B are also associated with certain cancers, but the majority develop as a result of genetic mutations, which makes finding a vaccine target somewhat harder. "The difficulty is that if there is no virus, there is nothing foreign for the immune system to recognise," says Professor Roy Bicknell, Head of the Cancer Research UK Angiogenesis Group at the University of Birmingham.

Attacking the support system

But that might yet be possible. Rather than second-guessing what mutations might someday arise in the body and vaccinating against them, Roy Bicknell is instead focusing his efforts on something all solid tumours need to grow: a blood supply. "We know that the blood vessels in tumours are structurally and genetically very different from those in healthy tissues," he says. For instance, he has identified four proteins that are highly expressed in the blood vessels of solid tumours. The same proteins are also produced by human embryos when they are first laying down a vascular system, but they don't seem to be made by healthy adults. "That potentially means we can attack them," Bicknell says.

His team has been developing CAR T cells against one of these proteins, called CLEC14a. But he is also working on a preventative vaccine that might destroy any blood vessels that a fledgling tumour begins to grow, therefore stopping it in its tracks. So far they've demonstrated that this is possible in mice.[4] "We have shown that if you vaccinate mice against the tumour vessels, then you get a strong anti-tumour effect," Bicknell says.

The real challenge with this, and other preventative cancer vaccines, will be proving that they work in humans. Most cancers take decades to develop; if you vaccinated subjects now, you'd have to wait a very long time to find out if the vaccine had actually prevented any cancers.

> *"History tells us that cancer is a shape-shifting enemy, and molecular therapies have been less successful than many had initially hoped"*

One approach might be to pick on the mutated proteins that drive the growth of cancer cells, such as the protein KRAS, which is implicated in 95% of pancreatic cancers. But such proteins are often found in the cytoplasm of cells, rather than on their surface. Immune cells can still mount a response to them, but it will be against small fragments of the protein, rather than the whole thing. This means targeting T cells, rather than antibody-producing B cells as conventional vaccines do. "T cells can see small protein changes within the cell; antibodies only see a whole protein," explains Professor Elizabeth Jaffee, Deputy Director of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University in Baltimore, USA.

She is developing a preventative cancer vaccine based on *Listeria*, a bacterium which grows and replicates inside human cells, using it to deliver proteins such as mutated KRAS to the antibody-presenting cells that show protein fragments to T cells. This sort of approach might work for cancers that are strongly associated with a specific mutation, such as pancreatic cancer. But for many cancers it's far harder to guess what the mutation might be, so it's unlikely to result in a universal cancer vaccine.

Moonshot challenge

To describe the goal of curing cancer with the immune system as a 'moonshot' is an understatement. The challenges are manifold, and if we ever do succeed it's likely to be the result of a combination of approaches – not all of them immunological – rather than a single one. But William Coley was right about one thing: given the correct stimulus, our bodies do have the capacity to reject cancer. We just have to learn the intricate sequence of buttons that need to be pressed.

1. Wiemann & Starnes 1994 *Pharmacology & Therapeutics* **64** 529–564
2. Van Pel & Boon 1982 *Proceedings of the National Academy of Sciences of the USA* **79** 4718–4722
3. Kalos *et al.* 2011 *Science Translational Medicine* **3** 95ra73
4. Zhuang *et al.* 2015 *Angiogenesis* **18** 83–95

Vaccination:
prevention is better than cure

Polio immunisation in Lucknow, India.

A hundred years ago, the death of a child from infectious disease was a common occurrence. Louis Pasteur, who took Edward Jenner's ideas about vaccination and suggested they could be applied to any microbial disease, lost three of his five children to typhoid – an illness that's now almost entirely preventable thanks to the technology he helped develop. Together with improved sanitation and antibiotics, vaccination has utterly transformed our relationship with pathogens. Smallpox has been eliminated, polio is on its way out, while other historic harbingers of death and debility such as measles and diphtheria are extremely rare. According to the World Health Organization, vaccines prevent around 3 million premature deaths per year.

"Vaccination is one of the most demonstrably effective and cost effective public health interventions that there is," says Andrew Pollard, Professor of Paediatric Infection and Immunity at the University of Oxford and Director of the Oxford Vaccines Group.

Challenges remain

However, despite the many success stories, effective vaccines against some of the world's biggest killers remain frustratingly elusive. Although the BCG vaccine against tuberculosis has been given to many millions of people, its ability to induce protective immunity varies between 18 and 80 percent depending on where you live. Even then, it only protects against severe childhood forms of tuberculosis; it does little to protect against the deadly and widespread adult lung infections. Similarly, RTS,S/AS01 – the most advanced vaccine being developed for malaria – is only 26 to 50 percent effective in infants and young children, even after four doses. And an effective vaccine against HIV remains a distant dream, despite more than 30 years of research and billions of pounds of investment.

In part, it's because the agents that cause these diseases are masters of disguise and immune system manipulation. However, a fundamental lack of understanding about how the immune system generates immunity has also hampered our best efforts. The good news is that powerful molecular tools are shedding new light on these processes, and this should ultimately lead to the development of better vaccines against these foes, and others.

Vaccinators in India immunising children against measles and marking it on their vaccination cards

History of vaccination

The practice of exposing people to a disease to protect them against future infection, known as variolation, can be traced as far back as 10th century China. Scabs from smallpox victims were either placed under the skin, or powdered and snorted up the nose to reduce an individual's chances of contracting smallpox. But it was Edward Jenner who drove the widespread use of this practice and in effect established the science of vaccinology as we know it today. He deliberately infected people with a less dangerous relative of smallpox called cowpox, and found that it protected them against future infection with both diseases.

Pasteur took this idea and developed it still further, reporting methods for attenuating the virulence of microbes so that they could be safely injected into the body and manufactured in bulk quantities for use around the world.

A hit and miss approach

The same principle underpins the development of pretty much every childhood vaccination we receive today. "You get a bug, you kill or inactivate it and then you inject the product into people – and if you're lucky it protects them against infection," says Peter Openshaw, President of the British Society for Immunology and Professor of Experimental Medicine at Imperial College London.

Incredibly, the transformative effects of vaccines on human and animal health occurred with barely any understanding of the immune events taking place in the body. "It was a hit and miss approach, but because there were so many attempts, it resulted in a large number of the vaccines which were partly responsible for the large decline in mortality from infectious disease during the second half of the twentieth century," Openshaw adds.

Although the development of most existing vaccines relied on trial and error rather than sophisticated immunology, we now know that the formation of this immunological memory involves distinct subsets of immune cells called B cells and T cells. Upon encountering the vaccine components (antigens), cells such as macrophages which specialise in processing and disposing of pathogens engulf the antigens and present them to B and T lymphocytes. The B cells churn out antibodies that protect against infection, while memory cells are also produced that will initiate a rapid response the next time that pathogen is encountered.

"Nearly every useful vaccine that's been developed to date acts through the production of antibodies," says Ronald Germain, Chief of the Lymphocyte Biology Section at the National Institute of Allergy and Infectious Diseases in Bethesda, USA.

The advantages of cellular immunity

However, this approach has taken us about as far as we can go. For one thing, the pathogens that cause diseases like tuberculosis, malaria, HIV, and many parasitic infections have all developed complex strategies to control our immune system, and evade detection – even hiding in our own immune cells in the case of HIV. "They control and work with our immune system, and against us," says Openshaw.

But antibodies alone don't seem to be enough. We also need T cell directed 'cellular immunity' in which our immune system is able to destroy cells that have already been infected by the pathogen. However current vaccines aren't very good at generating this type of immunity. "Unfortunately, we have not learned yet how to make vaccines that operate at the cell-mediated level in a highly effective manner," says Germain.

One strategy currently being investigated is DNA vaccines. Here, small pieces of DNA encoding antigens from the virus are inserted into a bacterial plasmid, which is then injected in the hope that some of our cells will take up the DNA and essentially become vaccine-antigen factories themselves – manufacturing and secreting the bit of the pathogen which the immune system can react to.

However, even if we could get our T cells to work better, it's unclear precisely which T cells we should be targeting and in which locations. A major issue is that no-one really knows what the immune system looks like in people who are protected against diseases such as TB. "If we had a measure or a correlate of protective immunity, we'd be able to go about the development of vaccines in a much more rational way," says Professor Ajit Lalvani, Chair in Infectious Diseases at Imperial College London.

Getting to know the immune system

So how do we go about getting better acquainted with our immune systems? The classic approach has been to focus on a single cell type, protein or signalling molecule at any given time. But technological advances now make it possible to rapidly combine multiple measurements of cells, tissues and blood, in order to build a fuller picture of how they work together to generate immunity. "By learning about what's happening in the immune system with these measurements, we can potentially see what we need to fix to make the response to vaccines better," says Germain. "It's a more rational approach to designing vaccines."

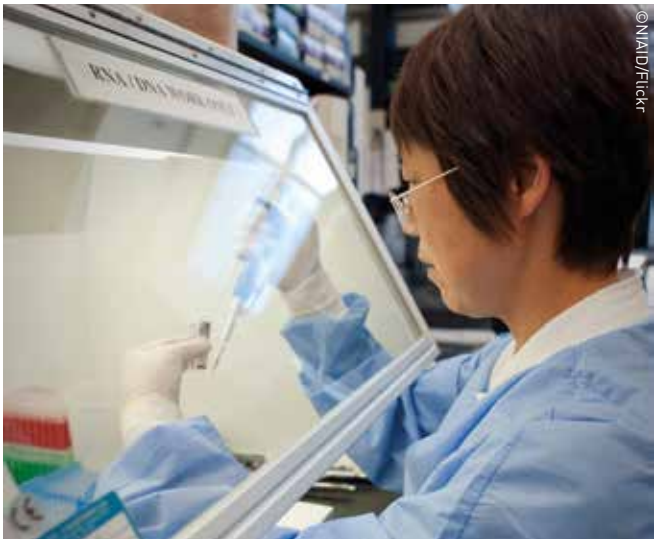
Already, it's paying off in the field of influenza research. Although vaccines against seasonal flu exist, the protection they afford is only short-term because the influenza virus is constantly evolving. In 2014, Germain and his colleagues announced that they'd identified an immunological signature which predicted how well people would respond to the seasonal and H1N1 flu vaccines.[1] To do so, they measured and compared the frequencies of different immune cell types, the expression of genes, the levels of flu-specific antibodies, and the activity of antibody-producing B cells in 60 volunteers both before and after they were vaccinated.

"These measurements are not related to influenza-specific immunity; they are broader measures of the immune system and they are reasonably stable in individuals over time," says Germain. "We can begin to distinguish who will be a high and a low responder, and having done that we can say, 'what is that telling us about the human immune system and is there something we can do to make the low responders better?'"

Lalvani's team have also identified a subset of T cells present in the blood of people who were exposed to the H1N1 virus during the 2009 flu pandemic, but didn't develop symptoms.[2] Knowing this, they are now looking at ways of stimulating the body to produce more of these cells.

Market insights

As well as enabling us to develop more effective vaccines, these kinds of insights might also reduce the amount of time it takes to bring new vaccines to market. At the moment, this typically takes around 12–15 years, a large part of which is taken up with field trials that involve injecting the vaccine into large numbers of people and waiting to see how many of them develop the disease. However, "if you could give the vaccine and two weeks later measure an immune response that told you it was going to work, then something which may currently take many years to develop could instead take just a couple of years," says Openshaw. "The trouble is that we still don't really know how vaccines work, or why something that should work doesn't."



The point of delivery

Understanding the type of immunity needed to protect people against hard-to-vaccinate diseases is only part of the challenge, however. Immunologists also need to figure out how best to generate that protection. For instance, the BCG vaccine against tuberculosis is usually injected into the arm, but the usual route of infection is inhalation through the lungs. It therefore makes sense to try and target immune cells living in the lining of the lungs, which might mean the creation of new types of vaccines, such as inhalable ones.

Researchers are also working on dissolvable skin patches as an alternative to traditional injected vaccines. Diseases like tuberculosis, malaria and HIV disproportionately affect people living in some of the world's poorest regions – places with poor transport infrastructure and limited access to electricity, which is needed to keep vaccines refrigerated. To this end, researchers are investigating delivering dried live vaccines through dissolvable polymer skin patches studded with tiny needles, which could be kept at room temperature and even be self-administered. Rather than injecting the vaccine into muscle tissue, the microneedles instead target antigen-presenting cells in the skin, but early results suggest the end result may be similar. For instance, when researchers at King's College London recently loaded a candidate HIV vaccine into such patches and applied them to the skin of mice, they recorded an immune response equivalent to when a liquid version of the vaccine was injected.[3]

Whatever the next hundred years holds, it's clear that twentieth century approach of growing a bug, disabling it and injecting it isn't going to be enough to rid the world of infectious disease and the misery it causes. Some of the pathogens we're fighting have been with us for millennia and know our immune systems far more intimately than we do, while others are newly-emerged and bring healthcare challenges all of their own. We'll have to at least match the knowledge and ingenuity of these pathogens if we're going to beat them.

1. Tsang *et al.* 2014 *Cell* **157** 499–513
2. Sridhar *et al.* 2013 *Nature Medicine* **19** 1305–1312
3. Bachy *et al.* 2013 *Proceedings of the National Academy of Sciences* **110** 3041–3046

Emerging threats: the evolving immunological response

"The recent Ebola outbreak was a shocking reminder of the threat we all face from a disease outbreak," said David Cameron on the eve of a G7 summit in June 2015. "We will face an outbreak like Ebola again and that virus could be more aggressive and difficult to contain. It is time to wake up to that threat."

The Prime Minister's words came as the Ebola epidemic that cost more than 11,300 lives in West Africa was coming to an end. Two months earlier, the Cabinet Office's National Risk Register of Civil Emergencies identified emerging infectious diseases as among the most serious threats facing the UK. Mr Cameron went on to outline plans for a new UK Vaccines Research and Development Network to coordinate national research efforts on some of the most threatening emerging risks including Ebola, Lassa fever, Marburg virus disease and Crimean-Congo fever.

UK's scientific strength

The recent political lead the UK has taken is pre-dated by a long history of world class science in this field. An All-Party Parliamentary Group on Health report published last year ranked UK research on infection and immunology as of the highest quality among G7 nations between 2010-14, as measured by impact, or the frequency of referencing of scientific papers in peer-reviewed journals.

This scientific strength came to the fore during the Ebola epidemic as UK immunologists made vital contributions to the race for a vaccine, yet sadly their efforts came too late to prevent the large-scale loss of life. In a report published in January, the House of Commons Science and Technology Select Committee argued the government response was too slow, emergency research was inadequately coordinated and a lack of domestic vaccine manufacturing capabilities makes the UK vulnerable. More positively, however, the response to the disease and ongoing advances in the field generally offer hope that we can improve our resilience to future emerging infectious diseases outbreaks.

Anatomy of the outbreak

The West Africa Ebola outbreak is believed to have started with the death of a young boy in Guinea at the end of 2013. New cases emerged among his family members, their contacts and healthcare workers. By the end of March 2014, the disease was identified as the deadly Zaire species of the

A volunteer taking part in the Ebola vaccine trial at the Jenner Institute

Ebola virus, and the infection had spread to nearby Liberia and Sierra Leone. The World Health Organization (WHO) announced the outbreak to be a public health emergency of international concern in August 2014, and set about fast-tracking clinical trials of two candidate Ebola vaccines.

Vaccine trials begin

Professor Adrian Hill, Director of the University of Oxford's Jenner Institute, agreed to lead a human trial of a chimpanzee adenovirus Ebola vaccine called ChAd3 EBOZ, previously developed by GSK and the US National Institutes of Health. Funding and regulatory approval were rapidly agreed, and the first healthy volunteers were vaccinated on 17 September 2014. "We started just over a month after being first contacted, which was unprecedented," says Hill.

The trial results, showing the vaccine generated an immune response and had an acceptable safety profile, were published in January 2015. During that year, further trials of both ChAd3 and other vaccines were launched. In July, the results of a large trial of the other existing vaccine, rVSV-ZEBOV, were published. Based on vesicular stomatitis Indiana virus, it was shown to offer at least short-term effective protection. By the time it was used, the numbers of new Ebola cases were rapidly decreasing. It is however believed that use of rVSV-ZEBOV hastened the end of the Ebola outbreak in Guinea.

Hill, who believes ChAd3 EBOZ is likely to offer more effective long-term protection, says that the hard truth is that there was no vaccine ready to use against Ebola at the start of the outbreak because it was not a commercial priority for the pharmaceutical industry. "We need to get on and put vaccines through clinical tests as well as making them," he says. "The wider lesson people are grappling with now is that there are probably another dozen outbreak pathogens for which it's relatively feasible to develop vaccines; however there just isn't a business case for doing so."

Had the Ebola outbreak occurred a decade earlier, it is unlikely that vaccines would have played a major role in the response. Vaccines based on adenovirus and VSV were not yet available, and vaccine manufacturing processes have also come a long way since then. Another technology that was central to the fight against Ebola, and which has developed rapidly in the last 10 years, is genetic sequencing.

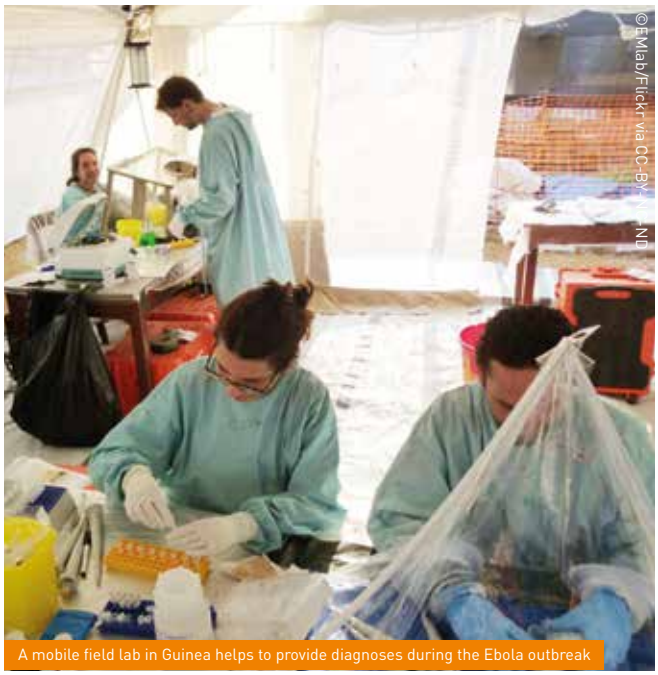
Know your enemy

The ability to quickly and accurately sequence pathogen samples has had major impacts on how we respond to emerging threats more generally. It can firstly identify pathogens, helping to reveal whether an outbreak has been caused by something previously known or entirely new. It can play a role in determining the sensitivity and specificity of diagnostic tests. When it comes to looking for treatments, genetic tests can reveal whether a virus or bacteria is related to other known threats, and therefore offer clues to drug susceptibility or resistance.

A key variable for epidemiologists dealing with an emerging threat is its 'basic reproductive number'. Also called R_0, this is the average number of infections one existing case generates. It can be calculated simply by counting the number of existing and new cases; however this is prone to error, and genetics can provide an alternative means of calculating R_0.

> *"An All-Party Parliamentary Group on Health report published last year ranked UK research on infection and immunology as of the highest quality among G7 nations between 2010-14"*

Once the virus's rate of mutation and the length of time that cases are symptomatic and infectious are known, genetic testing can reveal useful details of transmission chains that can help shape infection control methods. US researchers who sequenced 99 samples of the Ebola virus were able to determine that it was spread from Guinea to Sierra Leone by 12 people who attended the same funeral. Genetic testing also demonstrated camel-to-human transmission of MERS coronavirus.



A mobile field lab in Guinea helps to provide diagnoses during the Ebola outbreak

Speed is of the essence

"We shouldn't give the impression that genetics is a panacea," says Paul Kellam, Professor of Virus Genomics at Imperial College London. "Nevertheless it is an important tool because it can speed up the understanding of patterns of transmission, and the quicker you can target public health measures or deploy interventions effectively the better."

Professor Peter Openshaw, of Imperial College and the President of the British Society for Immunology, agrees. "Speed is absolutely of the essence in infection control. If sequencing can allow you to intervene two weeks earlier, you could prevent an exponential growth in cases, which might prevent hundreds of thousands of people being infected or needing to be put in quarantine."

Genetic testing's potential value has in the past been held back by the need to transport samples from sometimes remote locations to laboratories equipped with large, expensive sequencers. During the Ebola outbreak, however, a DNA sequencer smaller than a mobile phone was used to reveal the unique genetic fingerprints of Ebola virus samples taken from patients in Guinea within 24 hours. The MinION, developed by Oxford Nanopore Technologies, works by passing DNA strands through proteins with holes at their cores. As the four chemical building blocks of DNA (known as bases) pass through the hole, they impede an electric current passing through it in a characteristic way, allowing them to be identified and therefore the molecule to be sequenced.

Data on DNA mutations in samples from Guinea was sent to microbiologist Dr Nick Loman and colleagues at the University of Birmingham for analysis, providing insights into the sources of cases. It helped confirm, for example, fears that the flow of people across the border with Sierra Leone was prolonging the outbreak, and facilitated the more effective targeting of resources to fight the epidemic. Loman now has Medical Research Council funding for the mobile collection of 750 Zika virus genomes in Brazil.

Know your own weaknesses

Just as it is possible to sequence pathogen samples from different people, genetics can also be used to identify immune system variations of individuals. B cells play a vital defensive role in flagging up the presence of invading pathogens to stimulate other immune cells to attack them. In recent years, researchers have developed immune repertoire sequencing to profile B cells present in healthy individuals, as well as those with infections and malignancies.

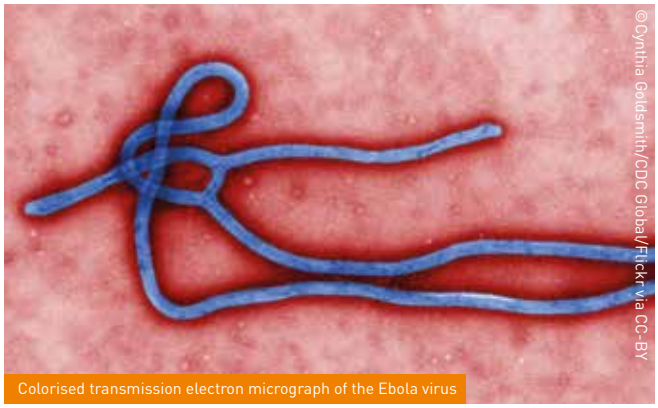
Dr Rachael Bashford-Rogers, of the University of Cambridge, has used the technique to profile changes in B cell populations as a result of treatment for chronic and acute lymphocytic leukaemia. She was able to detect the small numbers of leukaemia cells that remain in patients following treatment to a high degree of accuracy. This is important as it is a strong predictor of relapse. Dr Dominic Kelly, at the University of Oxford, is using the technique to study B cell responses to hepatitis B and influenza infections. It has also been used in a similar way to study dengue fever. The hope is that the technique will lead to the development of improved vaccines, diagnostics and treatments for emerging infections and other conditions.

Individual variations

Another application of genomics is in providing greater insight into the role of human genetic variability on disease severity. Within a given human population infected with a pathogen, some may become severely ill and die, while others experience only mild symptoms or may even not know they have the infection. Researchers have shown that in many cases variation in human genetics is more significant in determining disease severity than pathogen genetic variability.

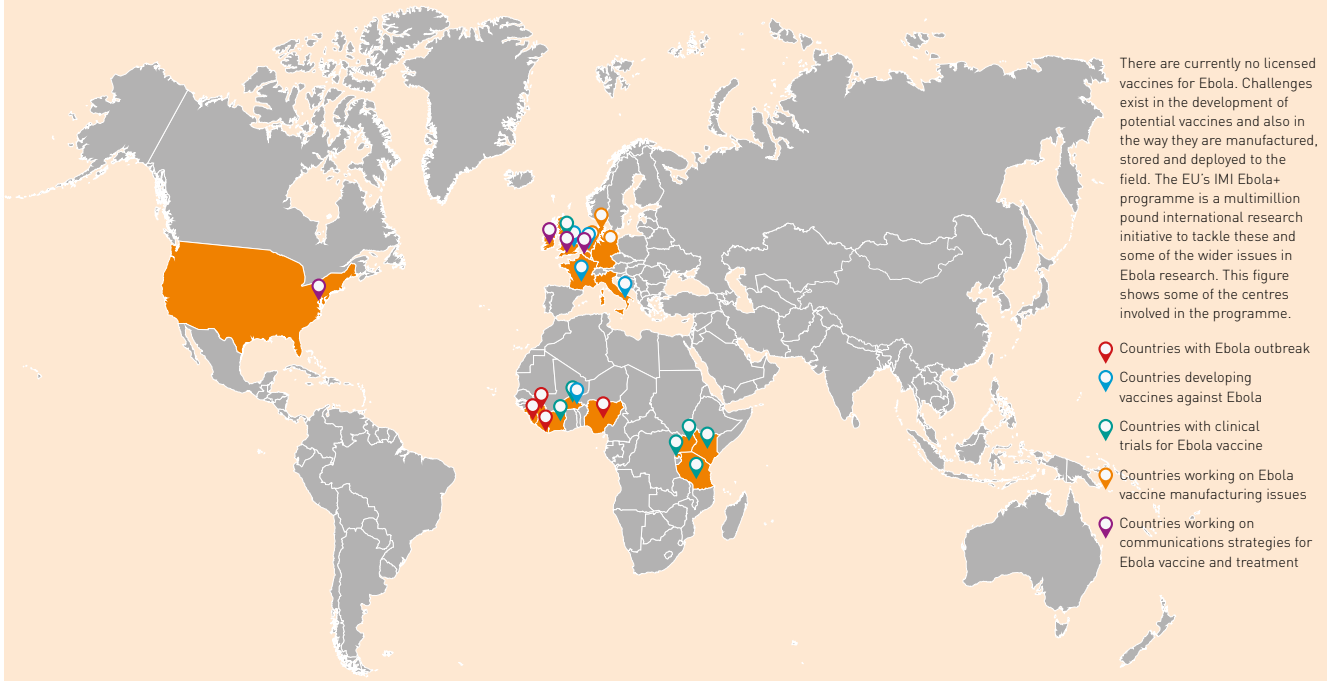
In 2012, Paul Kellam's group at the Virus Genomics lab at the Wellcome Trust Sanger Institute discovered that different variants of the human gene *IFITM3*, which encodes a protein that can make it harder for viruses to penetrate cells and replicate, are a key determinant of disease severity in influenza A (H1N1) patients. Research has also shown that variants of a gene that encodes the protein CCR5 can protect against HIV-1 by making it harder for the virus to penetrate target cells – Pfizer's HIV drug Maraviroc is based on this work.

"This offers us a new paradigm for the future of genetics," says Kellam. "If you can find a genetic variation in the human host that protects from a pathogen and does not cause any negative effects, this suggests a target for drugs to prevent infection."



Colorised transmission electron micrograph of the Ebola virus

DEVELOPING VACCINES AGAINST EBOLA - A GLOBAL EFFORT



Effective use of data

While researchers are now better able to collect information during emergency outbreak situations, what really counts is how that data is used. "The best patterns emerge when you cross compare everybody's data," says Kellam, "and that means being willing to share it openly and rapidly."

The WHO and Wellcome Trust have emphasised the importance of finding ways to encourage the sharing of data, while respecting patient confidentiality. Researchers leading the way in this endeavour include Dr Richard Neher of the Max Planck Institute for Developmental Biology in Germany, and Dr Trevor Bedford of the Fred Hutchinson Cancer Research Center in the USA who have developed websites that generate real-time visualisations of seasonal influenza and Ebola virus evolution. Professor Andrew Rambaut of the University of Edinburgh collates outbreak data and blogs about it.

> *"Speed is absolutely of the essence in infection control. If sequencing can allow you to intervene two weeks earlier, you could prevent hundreds of thousands of people being infected or being put in quarantine"*


An Ebola test lab in Liberia

Preventing future outbreaks

Hill believes that beyond developing and testing vaccines for the most dangerous emerging infectious diseases, two strategies offer hope for preventing outbreaks. First, small quantities of vaccines should be stockpiled in the locations in which infections are most likely to occur. Secondly, healthcare workers and first responders should be protected with routine vaccinations. "It would protect them of course, but it could also stop outbreaks from getting going because doctors and nurses often play key roles in spreading infectious pathogens," says Hill.

Those who highlight the tragedy of the loss of thousands of lives as a result of the failure to carry out clinical trials of candidate vaccines that existed before the West Africa Ebola outbreak are right to do so. Yet if the right lessons can be learnt, and real progress be made in key areas like rapid mobile genetic sequencing, immune repertoire sequencing, understanding the role of genetic variability in human hosts and in data sharing, we can at least improve humanity's odds in the inevitable battles with emerging infectious pathogens that tomorrow will bring.

The British Society for Immunology's mission is to promote excellence in immunological research, scholarship and clinical practice in order to improve human and animal health.

We are grateful to all the people who agreed to be interviewed for this report.

This report was authored by Nic Fleming and Linda Geddes.

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