Sir Macfarlane Burnet receiving Nobel Prize for Medicine or Physiology, Stockholm, December 1960. Photo courtesy WEHI

One POWERFUL Idea

This month marks the 50th anniversary of Sir Macfarlane Burnet's publication of his ground-breaking "clonal selection theory" in *The Australian Journal of Science* – which is now published as *Australasian Science*. Stephen Turner explains the impacts of Burnet's theory on the consequent and ongoing discoveries made in immunology.

I have been recently involved in helping to organise a meeting that celebrates the achievements of probably one of the greatest immunologists of last century, Sir (Frank) Macfarlane Burnet. The focus of this meeting is not the work that resulted in Burnet's 1960 Nobel Prize in Medicine or Physiology, but the 50th anniversary of his publication of the "clonal selection theory".

As a young research scientist working in the field of immunology, I enjoy the benefits of advanced cellular and molecular techniques that enable me to conduct experiments with exquisite sensitivity and detail. It is amazing to consider that many of the principles I now take for granted were laid out by Burnet 50 years ago.

Burnet first presented his theory in a two-page essay that predicted the cellular and molecular mechanisms that result in the induction of immune responses to infection. He set about predicting the nature of our immune system that allows it to recognise and protect us from the vast array of pathogens we are exposed to daily. It was not until the advent of the advanced techniques I now take for granted that Burnet's theories would be proved essentially correct 30 years later.

Modern immunology started with Burnet's clonal selection theory, and he started a new field of research with its publication in *The Australian Journal of Science*, which is now published as *Australasian Science*. Importantly, Burnet helped establish the excellent international reputation of Australian immunological research that my colleagues and I still benefit from today.

While many of Burnet's predictions have turned out to be correct, our increased knowledge has also led to many new questions and the realisation that we don't have all the answers. Finding answers challenges us in trying to develop new strategies for treating or preventing diseases such as HIV/AIDS, malaria, multiple sclerosis or allergic reactions.

Why Burnet Is Famous

Burnet was awarded the 1960 Nobel Prize for the theory of acquired immunological tolerance he developed from 1949. Burnet, also an accomplished virologist and microbiologist, realised that our immune systems must be capable of recognising pathogen-specific chemical signals when we are infected and mobilise our immune defence systems quickly.

Importantly, he realised that many of these chemical signals are present in our own tissues. He theorised that our immune systems are educated to ignore our own tissue ("self") and to recognise foreign chemical signals ("non-self"). He predicted that this state of immunological tolerance occurred during foetal development.

Sir Peter Medawar, with whom Burnet shared the Prize, confirmed Burnet's theory experimentally. Burnet's and Medawar's work had enormous implications for medicine by explaining why tissue rejection occurs after organ transplantation. In this case, the donated tissue is seen as non-self or "foreign" by the recipient's immune system.

What made this finding impressive was that Burnet formulated his theory without the advantage of the advanced technology of today. It was purely and simply a theory based on observation,

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A Modification of Jerne's Theory of Antibody Production using the Concept of Clonal Selection

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In 1957 Burnet insisted on publishing his clonal selection theory in the peer-reviewed *Australian Journal of Science* – now published as *Australasian Science* – thereby giving prominence to the theory's Australian origins.

logic and incredible insight.

While this work was surely deserving of a Nobel Prize, it is not his only work with a significant impact on our understanding of the immune system. It can be argued that Burnet probably deserved a second Nobel Prize for his 1957 theory of clonal selection.

Acknowledging the Work of Others

As scientists, the ideas and theories we formulate are shaped to some extent by the ideas and theories of our predecessors and colleagues. The paper Burnet published in the 21 October 1957 edition of *The Australian Journal of Science* starts, like all good scientific papers, with the historical background and rationale for his thinking by acknowledging the theories of Niels K. Jerne (winner of the 1984 Nobel Prize) and David Talmage.

Jerne first postulated that our body had a vast array of naturally occurring molecules, termed "antibodies". Upon infection, only those antibodies with the appropriate shape for the invading microbe could bind to it and negate its effect. However, Jerne's theory lacked a reasonable explanation for how large amounts of "specific antibody" could be generated after infection.

Burnet agreed with Jerne that, when a foreign component enters the body, it attaches to a single antibody that has the appropriate shape for contact. Think of this like two jigsaw pieces fitting together.

Dr Macfarlane Burnet at the Walter & Eliza Hall Institute for Medical Research in the mid-1950s. Photo: WEHI

There is a vast array of unique antibodies present, each with its own special shape. This provides a library of unique receptors capable of binding to the many different shapes that would be found in "foreign biological material" such as microbes.

In early 1957 Talmage published a review of theories of antibody production that strongly supported Jerne's idea. However, he proposed that selecting and expanding cells might be the mechanism for replicating specific antibody. He forwarded his manuscript to Burnet, who had independently formulated a similar theory.

Talmage's paper seems to have spurred Burnet to commit his ideas to test. Interestingly, Burnet noted receipt of Talmage's manuscript in his paper. It's a terrific lesson for young scientists that acknowledging the contribution of others is important for ensuring credibility in research.

Predictive Power

In contrast to Jerne, Burnet (and Talmage) predicted that contact between the foreign component and the antibody must occur on the surface of an immune cell (termed "lymphocyte"). Therefore, the body must contain an army of lymphocytes "expressing" (making) a unique antibody

Three scientific generations of Australian scientists whose work benefits from Burnet's theory. At the University of Melbourne, Nobel Laureate Professor Peter Doherty (middle), Dr Stephen Turner (right) and PhD student Lauren Hatton examine the results of a "plaque assay" that measures the infectivity of an influenza virus. Doherty and Turner are researching how our immune systems fight virus infections and establish immunity. The assay demonstrated that, by increasing the number of immune cells utilising a novel vaccine strategy, the infectivity of influenza A virus was reduced. Photo courtesy Stephen Turner

receptor. The lymphocyte would then produce this specific antibody that could then bind and negate the infection.

What set Burnet's theory apart was that he predicted how the immune system must be "wired" for his theory to work. For example, he predicted that, upon contact between the foreign component and the surface-bound antibody, the lymphocyte would settle in an appropriate tissue, such as a lymph node. There the lymphocyte proliferates, generating a large number of clones, each expressing the same antibody originally selected.

Among other predictions that made his theory all the more remarkable was he recognised that to generate this "preformed" library of lymphocytes, each expressing a unique antibody receptor, the genes within each lymphocyte must be able to introduce random mutations into their own unique receptor DNA sequence. Each lymphocyte would have a unique gene sequence encoding a unique antibody receptor. This would ensure that the appropriate lymphocyte could be called upon when required (i.e. when we are infected) to produce antibody and negate the infection.

Secondly Burnet realised that, with this randomisation, antibody receptors could be generated that recognise components from our own body (self). Therefore he predicted that during foetal development these "self-reactive" lymphocytes would need to be eliminated.

This sat nicely with his prior theory of immunological tolerance. With his clonal selection theory he had provided the mechanism explaining how immunological tolerance could work. This aspect of the theory was also tested experimentally and shown to be correct.

A Blueprint for Modern Immunology

Burnet wanted his theory to be a blueprint for future research. He figured "it has so many implications calling for experimental enquiry" that it warranted publication. It is remarkable how much of Burnet's theory stood up to rigorous experimental enquiry in the next three decades.

We now know that the lymphocytes Burnet referred to are specialised cells of the immune system that we call "B cells". They express cell surface receptors (antibodies) that bind to foreign components entering the body and result in the cascade of events described earlier.

Even more impressive was that his predictions regarding the organisation of the immune system also proved correct. For example, the genes encoding antibody receptors can generate random shapes. However, the mechanism remained a mystery until the advent of advanced molecular biology some 30 years later.

Additional consequences Burnet predicted from his clonal selection theory (including the production of soluble antibody and the establishment of long-lived memory cells) were also proved correct with time. These consequences of successful "clonal selection" are what we try to achieve with vaccines that trick our immune systems into thinking we are infected in order to establish immunity.

Burnet's theory touched most fields of modern research in immunology, and his blueprint initiated many active projects. For example, in my laboratory we study how our immune systems establish "immunological memory", resulting in protection from a secondary infection.

Another outcome was the impact Burnet's theory had on establishing Australian researchers as world leaders in the field. By publishing his theory in an Australian science journal, he reasoned if the theory was correct it would emerge as important globally and he would be given credit for the ideas. Alternatively, he thought that if it was proved wrong (or worse, crazy), his overseas colleagues wouldn't readily see it and he would avoid embarrassment.

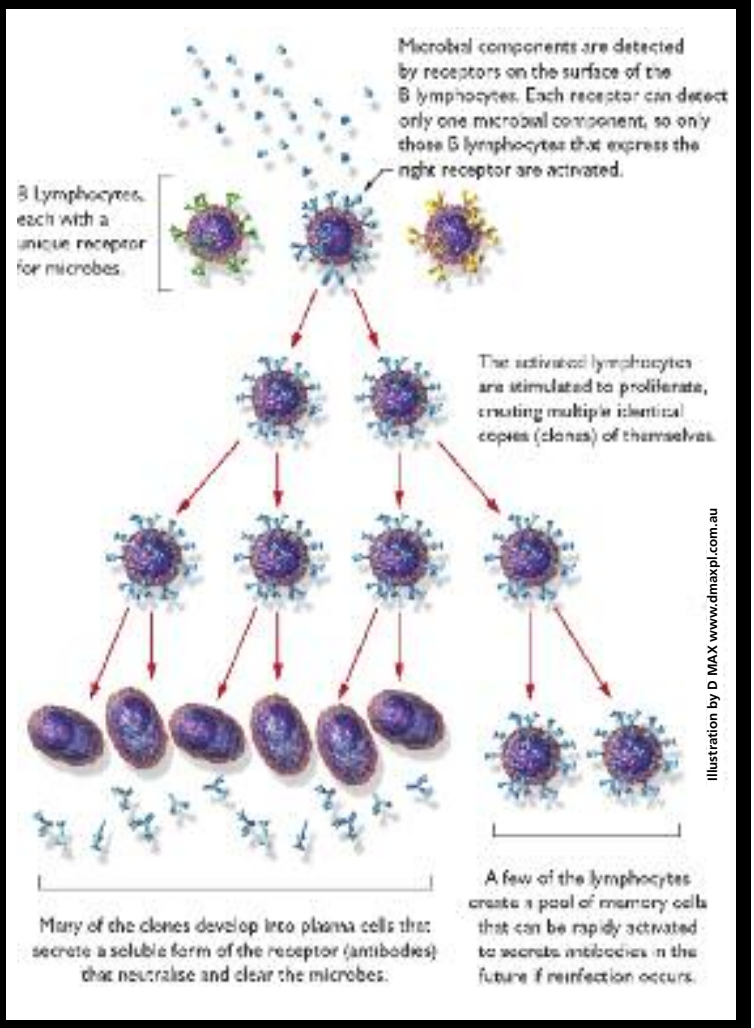
Burnet's Theory Explained

Burnet designed his theory to explain how our immune systems are able to fight off infections in a specific manner. Microbes that cause infections, such as bacteria or viruses, are made up of complex protein structures. The immune system targets small sections, or components, of these microbial protein structures.

He predicted that our immune systems contain a vast number of immune cells (called B lymphocytes), each with a unique cell surface receptor that is specific for a single microbial component. Upon infection (whether it be bacterial or viral), a small number of B lymphocytes will recognise their unique microbial component.

These B lymphocytes are selected from a vast array of unique lymphocytes to fight the infection. Recognition of a specific microbial component by the B lymphocyte results in the cell being activated. There are three main consequences of this activation:

1. The B lymphocytes are stimulated to proliferate, making identical versions of themselves (clones);
2. a proportion of these B lymphocyte clones start to produce vast amounts of a soluble form of the microbial-specific receptors (called antibodies). These antibodies bind to the microbe to subdue and eventually clear the infection; and
3. a small proportion of these B lymphocyte clones will persist after the infection clears. In the event of a second infection by the same microbe, these "memory" B lymphocytes can be reactivated to produce antibody more quickly, thus helping to protect a person from a second infection. The generation of memory B lymphocytes forms the basis of immunity to infection. Vaccination is a way of inducing memory lymphocytes without having first to be infected.



Burnet's position as head of the Walter and Eliza Hall Institute enabled him to inspire other Australian researchers to test and explore the new theory. This gave them a head start on overseas competitors. For example, (later Sir) Gustav Nossal, mentored by Burnet at the Walter and Eliza Hall Institute, experimentally confirmed both the theory that a single lymphocyte produced a single antibody molecule and that lymphocytes with receptors reacting with "self" were inactivated.

Burnet's clonal selection theory helped establish a climate for research in Australian immunology that still enjoys an international reputation for training scientists of the highest calibre.

What Burnet's Theory Did Not Predict

With advanced genetic, biochemical and physical techniques, we can now dissect the events that regulate immunity to infection with a detail that not even Burnet could have imagined. For example it is now known that there is a second set of lymphocytes called "T cells". Just like B cells, T cells have a cell surface receptor that can recognise foreign components, but they don't produce antibody.

Burnet did allude to a subset of cells that caused immune reactions without making antibody, likely to be the later-discovered T cells. What Burnet couldn't predict is that T cells don't "see" free foreign components like B cells. T cells see foreign components that have been first processed and then presented at the cell surface associated with host proteins called "major histocompatibility complex (MHC) proteins".

Significantly, T cells from an individual only see these foreign components when they are presented by their own MHC. Professors Peter Doherty and Rolf Zinkernagel first described this phenomenon, termed "MHC restriction", while working at the John Curtin School of Medical Research, and were jointly awarded the 1996 Nobel Prize for their discovery. (I have had the privilege of working with Doherty since 1999.)

Other aspects of immunity not predicted by Burnet included the role

Burnet's bust at WEHI by A. Bonett.

that the innate immune system (present at birth) plays in helping to initiate immune responses. Cells of the innate immune system don't have the specific receptors associated with T or B cells. Therefore, the innate immune system cannot generate specific immunity or immunological memory. However, our innate immune system plays an important role in protecting us from infection as it is our first line of defence.

The innate immune system buys time for both B and T cells to get going and produce the number of specific lymphocytes required to protect against infection and establish immunity. It also acts as an alarm to alert the T and B cells to an infection. This innate alarm system, present in many species including flies and reptiles, recognises the presence of particular microbial elements such as viral genetic material or bacterial cell wall components. Detection of these elements sends a "danger signal" to the immune system and triggers activation of the appropriate lymphocytes.

Challenges for Modern Immunology

Burnet's blueprint of specific immunity to infection has diminished the impact of many infectious diseases. Vaccination is based on activating microbe-specific lymphocytes into thinking we are being infected, resulting in immunity. Using this strategy, smallpox has been eradicated from the globe, eradication of the polio virus is imminent and we have effective vaccines against diphtheria, hepatitis B, tetanus, yellow fever, measles and typhoid.

However, major challenges remain. For example, we don't have effective vaccines against major human infectious diseases caused by human immunodeficiency virus (HIV, the causative agent of AIDS) or the parasite *Plasmodium falciparum* (the cause of malaria). One reason is that we are only now appreciating the mechanisms utilised by these microorganisms to evade immune mechanisms.

We also need to learn more about what it takes to generate robust and effective immunity. For example, my area of research examines how immune lymphocytes become long-lived memory cells. Despite Burnet predicting this 50 years ago, this area of immunology is still a big mystery.

Such challenges will require innovative strategies. Our increasing understanding of how the immune system generates specific immunity means we can improve current vaccine strategies and design new ones. A recent success is Prof Ian Frazer's vaccine against human papilloma virus, a causative agent of cervical cancer. Rather than use an altered version of the virus (like the polio vaccine), his vaccine uses a novel technology, called a virus-like particle, that looks like a virus but is not infectious.

Improving vaccines includes making them cheaper and more stable. This is important if we are to provide effective vaccines to the developing world where diseases such as HIV/AIDS and malaria have their greatest impact.

We are only now just starting to understand the complex pathways involved in diseases where the immune response has gone awry, such as multiple sclerosis or Type I diabetes. Australian researchers, such as Prof Len Harrison, are leading the way in the treatment of diabetes using a vaccine strategy that inactivates the self-reactive lymphocytes causing the disease. This strategy of disease intervention can be predicted based on Burnet's blueprint.

As scientists in this modern day and age, we have a fantastic array of tools at our disposal enabling us to understand many aspects of immunity at greater depth than previously thought. It is humbling to think that the powerful framework we utilise to gain this knowledge, and develop novel vaccines and disease interventions, was laid out in two pages by Burnet 50 years ago.

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Sir Macfarlane Burnet (right) and Professor Gustav Nossal, his successor as Director of the Walter & Eliza Hall Institute of Medical Research, ca 1965. Photo: WEHI