

A scientific odyssey: unravelling the secrets of the thymus

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My early research career has benefited greatly from chance and the right environment. It was not my intention to solve major problems in my postgraduate studies. I was simply interested in the possibility that a virus, discovered by Ludwik Gross in the United States, was involved in the pathogenesis of mouse leukaemia. Although I had no plans to work on immunological problems, I was very much influenced by lectures given by two giants in medical research — Peter Medawar (who, along with Macfarlane Burnet, was awarded the 1960 Nobel Prize for physiology and medicine for their contributions to immunology) and James Gowans (who was Professor of Experimental Pathology at Oxford). Both were responsible for elucidating the phenomenon of immunological tolerance and the function of recirculating small lymphocytes. Their expositions helped me greatly in my subsequent work on the immune system.

Early years and how I ended up in medical research

My parents were born in Paris in 1896. During the first World War, my father Maurice Meunier (English translation, Miller) was an interpreter for British forces in France. In 1919, he married and left for China to join a French bank in Peking. He spent some 22 years in China and Japan, eventually becoming manager of the Franco-Chinese Bank in Shanghai. In 1930, my mother (for health reasons) and my two sisters returned to France by boat. My mother found that she was pregnant, so, having been conceived in China, I was born in Nice, France, in April 1931. The following year we (my mother, my eldest sister Jacqueline, Jeanine and I) returned to China, but we were back in France again 3 years later, because of my mother's delicate health, and to allow Jacqueline to receive a "good" education at a French boarding school. One year later, when we were on the verge of returning to China, Jacqueline was diagnosed with pulmonary tuberculosis. The four of us instead went to live in Lausanne, Switzerland, which at that time was the place where tuberculosis was supposed to be cured. On the outbreak of World War II, the family hurriedly moved back to Shanghai where, unfortunately, Jacqueline had a relapse of her disease and died in December 1940, aged 17, sadly a few years before the discovery of the anti-tuberculosis drug, streptomycin.

In 1941, my father, believing that Japan would enter the war, decided that we should move to Australia. We took the last available cargo ship out of Shanghai and arrived in Sydney a few weeks before Pearl Harbour was attacked.

From an early age, and having witnessed my sister's illness, I wished I could study medicine. Even though I was 10 years younger than Jacqueline, I remembered well her doctor telling my mother how little was known about the body's resistance to infection, and that intrigued me. In Sydney I went to a Jesuit school, St Aloysius.

There, I became friendly with an Austrian boy from Vienna, Gus Nossal. Our paths were to cross in later years.

Having achieved a maximum pass in my last year at school, I was accepted into Sydney University medical school. In those days, the course lasted 6 years, but after the 4th year, I did a year's research. I pursued a Bachelors degree in medical science in Professor de Burgh's bacteriology department at Sydney University, investigating an experimental model of virus infection. This experience gave me a taste for basic research.

PhD studies: virus-induced mouse leukaemia

After passing my finals and doing an internship at the Royal Prince Alfred Hospital in Sydney, in 1957 I applied for a Gaggin Research Fellowship, advertised in *The Medical Journal of Australia*. It was sponsored by the University of Queensland, and offered a return fare to the United Kingdom and a salary for 2 years in a research institute. With this Fellowship in hand, I was accepted as a postdoctoral student for the PhD degree at the Chester Beatty Research Institute in South Kensington, London. I arrived in 1958 with no clear idea of what I might be doing. Many of the scientists at the Chester Beatty were involved in searching for new chemical carcinogenic compounds. Adding more compounds to an ever-growing list did not particularly interest me, as I preferred to use the experience I gained in my BMedSci year to work on some model in which pathogenetic mechanisms had to be elucidated. There was no space for me in the London laboratories, and I was sent to one of the two Chester Beatty satellites, "Pollards Wood", in Buckinghamshire. It had a splendid Tudor-style mansion in which the rooms had been refurbished to first-class laboratories and offices. There, Dr RJC Harris was working on the development of sarcomas in turkeys, induced by the Rous sarcoma virus. This interested me. Instead of working in his group, he suggested that I might investigate the pathogenesis of lymphoblastic leukaemia induced in mice by what was presumed to be a virus recently discovered by Ludwik Gross. I was very pleased to do this. The only space available was in a shack and in part of a converted horse stable near other horse stables that had been converted to animal holding rooms. Six months after I arrived, Harris was offered the directorship of the Division of Virology of the Imperial Cancer Research Fund at Mill Hill, London. He packed up and left, leaving me without an immediate supervisor, but it was some consolation to acquire his animal space.

In the late 1950s, Gross (at the Cancer Research Unit, Veterans Administration Medical Center, the Bronx, New York) had induced leukaemia in some "low-leukaemic" strains of mice by simply inoculating newborn mice with filtered extracts of leukaemic tissues from "high-leukaemic" strains of mice that spontaneously develop the disease at around 9 months of age. Repeating Gross's observations using the Pollards Wood strains of mice might have taken months or years, and so I wrote to Gross asking him whether he would be kind enough to send his virus and the mice harbouring it. I was grateful that he did so.

It was known at that time that acute lymphoblastic leukaemia in mice somehow involved the thymus, and that adult thymectomy prevented the disease from developing spontaneously in high-leukaemic-strain mice, and from induction by ionising radiation and



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chemical carcinogens in low-leukaemic-strain mice. As no one had hitherto investigated the role of the thymus in the leukaemia induced by Gross's method, I thought that this would be a good topic for my PhD studies. I had many questions to answer. Why did leukaemia develop only when mice were inoculated with the virus at birth and not later? Could adult thymectomy prevent the disease in virus-inoculated mice? Could the virus multiply only in thymus tissue? What would happen if a normal thymus was grafted into virus-inoculated mice that had their own thymus surgically resected? I was soon able to confirm Gross's initial work and, in addition, showed the following:

- Mice given the virus at birth did not develop leukaemia when thymectomised after weaning, but did develop leukaemia when grafted in later life with normal thymus tissue.
- Grafting normal thymus as late as 6 months after adult thymectomy still enabled leukaemia to develop.
- The virus could be recovered from the healthy non-leukaemic tissues of neonatally inoculated mice that had been thymectomised at around 6 weeks of age.

All these findings led me to wonder whether the virus could multiply in tissues other than the thymus, and to test whether day-old mice inoculated with virus after neonatal thymectomy would develop leukaemia when grafted 2–4 months later with thymus tissue.

From leukaemia studies to immune deficiency

Neonatally thymectomised mice grew well at first, but after weaning, many lost weight and died prematurely whether inoculated with virus or not. Adult thymectomy, on the other hand, had never shown any untoward effects such as weight loss, immune deficiency or obvious abnormalities. The onset of wasting and premature death after neonatal thymectomy led me to conclude, "that the thymus at birth may be essential to life".¹ Histological examination of the tissues of neonatally thymectomised mice showed a marked deficiency of lymphocytes in the circulation and the lymphoid tissues, and many wasted mice had liver lesions suggesting infection by a hepatitis virus. I might not have followed up these results had I not been aware of the brilliant work of two famous immunologists, Jim Gowans and Peter Medawar. Gowans had recently shown that, unlike thymus lymphocytes, circulating small lymphocytes were not short-lived cells, as had been widely believed — they were long-lived immunocompetent cells, recirculating from blood through lymphoid tissues into lymph and well equipped to initiate immunological reactions when appropriately stimulated by antigen. Medawar and his colleagues had proven that these lymphocytes were involved in rejection of foreign tissues. Clearly, my neonatally thymectomised mice, that had so few lymphocytes, must have been immunodeficient. So, I tested their immune competence by grafting skin from foreign mice and from rats and by testing their antibody responses to several antigens. The results were striking; unlike mice thymectomised as adults, which had been shown by many to be perfectly able to mount all types of immune responses, my neonatally thymectomised mice were immuno-incompetent. I concluded that during embryogenesis, the thymus would produce the originators of immunologically competent cells, many of which would have migrated to other sites at about the time of birth. This would suggest that thymus lymphocytes leaving the thymus are specially selected cells.^{2,3}

As had to be expected, thymus grafting restored immunological potential to thymectomised mice, but when the thymus donor was

foreign to the host, the latter was specifically tolerant of the donor's tissue antigens. I therefore suggested that tolerance is established within the thymus by the deletion of potentially reactive cells ("selective immunological thymectomy").³

I next turned my attention to adult thymectomy. It seemed to me that, as total body irradiation damaged the lymphoid system and its immune function, recovery following irradiation should be thymus-dependent. This was found to be correct.⁴

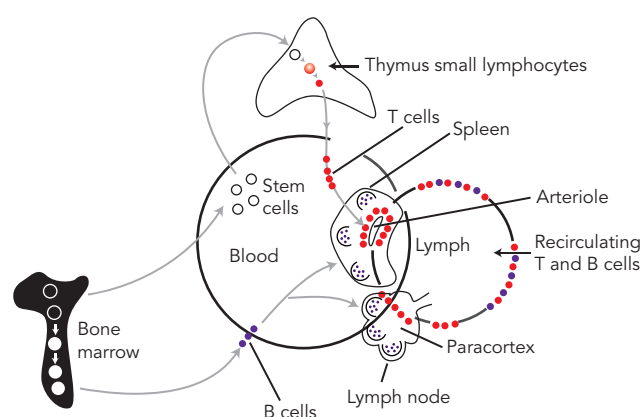
In those days, most immunologists considered the thymus a useless organ that had become obsolete during the course of evolution and acted simply as a graveyard for dying lymphocytes. They could not fault my data, but criticised my interpretation. The most valid criticism was that my mice, having been bred in converted horse stables, must have had so many intercurrent infections that the additional trauma of thymectomy made them immuno-incompetent. I was able to silence this criticism in 1963, when I obtained an Eleanor Roosevelt Fellowship enabling me to spend a year at the National Institutes of Health in the United States, at that time the only country where germfree mice were available. As neonatally thymectomised germfree mice were also immuno-incompetent,⁵ immunologists finally agreed that the thymus did have an immune function. However, it was not clear whether it produced all the body's lymphocytes, then widely believed to function as a single homogeneous population, or whether it influenced the lymphoid system by some unidentified humoral mechanism.

Back in Australia

In 1965, I was invited back to Australia by Gus Nossal, who had just been appointed director of the Walter and Eliza Hall Institute of Medical Research in Melbourne, to succeed Burnet. I was to lead a new laboratory at the Institute, and Gus had kindly chosen the brilliant young Graham Mitchell, who had just graduated with first class honours from the University of Sydney Veterinary School, as my first PhD student. Our first task was to understand how the thymus contributed to the pool of immunocompetent recirculating small lymphocytes. To achieve this, we investigated how various cell types might restore immune functions to thymectomised mice and what happened to these cells. At that time no "CD" (cluster designation) markers were available to trace cells, and the fluorescent-activated cell sorter had not been invented. We therefore used genetic markers (H-2 disparate strains of mice) and anti-H-2 sera to follow the fate of the injected cells. By the use of such markers, we were able to show how thymus-derived cells and bone-marrow-derived cells interacted in the formation of antibodies (Box 1).⁶⁻⁹ The data in these classical papers established unequivocally for the first time that: (i) thymus-derived cells (later known as T cells, short for "thymus-derived" and coined by Ivan Roitt in London) could be activated *specifically* by antigen; (ii) they were *not* the precursors of antibody-forming cells; (iii) they were essential to *help*, through some form of collaboration, other lymphocytes derived from bone marrow (later known as B cells, short for "bone marrow-derived" cells, also coined by Ivan Roitt) to respond to antigen by producing antibody; and (iv) the mammalian equivalent of the avian bursa (that had been shown by various investigators to have a unique function in antibody formation) was the bone marrow. As to the mechanism of interaction, I made various suggestions, notably that T cells would "focus" cells' antigen onto specific B cells, or that T cells might secrete antigen-non-specific pharmacological agents.

How did the immunological community react to our findings? There was complete surprise, of course, but there was also disbelief

1 Development and migration of T and B cells



Haemopoietic stem cells originate in the bone marrow (and in the yolk sac and liver in the fetus) and are disseminated in the bloodstream. Some that have already differentiated to lymphoid stem cells reach the thymus where they differentiate to T lymphocytes that migrate out to circulate in the blood and lymph and to colonise the T-cell-dependent areas of the lymphoid tissues. B cells differentiate in the bone marrow and migrate out to colonise the B-cell-dependent areas of the lymphoid tissues and to circulate in blood and lymph. ♦

2 Principal awards received

- Encyclopaedia Britannica (Australia) Award, 1966
- Gairdner Foundation Annual International Award; Toronto, Canada, 1966
- Scientific Medal of the Zoological Society of London, 1967
- Paul Ehrlich-Ludwig Darmstaedter Prize; Frankfurt, Germany, 1974
- Rabbi Shai Shacknai Memorial Prize; Jerusalem, Israel, 1978
- Elected Foreign Associate for the United States National Academy of Science, 1982
- International St Vincent Prize; World Health Organization, 1983
- Sandoz Prize for Immunology, 1990
- Peter Medawar Prize for the Transplantation Society, 1990
- Croonian Prize, Royal Society; London, 1992
- J Alwyn Taylor International Prize for Medicine; London, Ontario, Canada, 1995
- Copley Medal, Royal Society; London, 2001
- Prime Minister's Prize for Science; Australia, 2003 ♦

such as transgenic technology, and novel experimental approaches, such as gene targeting, that have given us so much new knowledge in immunology. Although we can employ numerous strategies to allow better survival of transplanted tissues, to deal with various forms of immunological aberrations, and to produce new vaccines, we still have much to learn, in particular, how to apply the fundamental knowledge obtained from our bench work in clinical situations. I am thus in full agreement with the scientific philosopher Karl Popper, that “the deeper our learning, the more conscious, specific and articulate will be our knowledge of what we do not know, our knowledge of our ignorance”.¹¹

References

- 1 Miller JFAP. Analysis of the thymus influence in leukaemogenesis. *Nature* 1961; 191: 248-249.
- 2 Miller JFAP. Immunological function of the thymus. *Lancet* 1961; 2: 748-749.
- 3 Miller JFAP. Effect of neonatal thymectomy on the immunological responsiveness of the mouse. *Proc Roy Soc* 1962; 156B: 415-428.
- 4 Miller JFAP. Immunological significance of the thymus of the adult mouse. *Nature* 1962; 195: 1318-1319.
- 5 McIntire KR, Sell S, Miller JFAP. Pathogenesis of the post-neonatal thymectomy wasting syndrome. *Nature* 1964; 204: 151-155.
- 6 Miller JFAP, Mitchell GF. The thymus and the precursors of antigen-reactive cells. *Nature* 1967; 216: 659-663.
- 7 Mitchell GF, Miller JFAP. Immunological activity of thymus and thoracic duct lymphocytes. *Proc Nat Acad Sci USA* 1968; 59: 296-303.
- 8 Miller JFAP, Mitchell GF. Cell to cell interaction in the immune response. I. Hemolysin-forming cells in neonatally thymectomized mice reconstituted with thymus or thoracic duct lymphocytes. *J Exp Med* 1968; 128: 801-820.
- 9 Mitchell GF, Miller JFAP. Cell to cell interaction in the immune response. II. The source of hemolysin-forming cells in irradiated mice given bone marrow and thymus or thoracic duct lymphocytes. *J Exp Med* 1968; 128: 821-837.
- 10 Burnet FM. Genes, dreams and realities. Oxford: Medical Technical Publishing, 1971.
- 11 Popper KR. Conjectures and refutations. The growth of scientific knowledge. 4th ed. London: Routledge, 1972.

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when I presented these results at meetings held in the US and Canada in 1968. I was accused of “complicating things”, but the commonest and quite valid criticism of our view of how T and B cells collaborated was that two rare clonally individuated cells would never find each other. The most sarcastic criticism came from Bede Morris, then the Professor of Immunology at the John Curtin School of Medical Research in Canberra, who likened B and T cells to the first and last letters of the word “bullshit”!

In spite of all this scepticism and criticism, Graham and I persevered in our work. It was urgently necessary to re-examine a multitude of immunological phenomena and diseases of immune aberration in terms of the two-cell system — tolerance, memory, autoimmunity, immune deficiency, genetically determined unresponsive states, mode of action of immunosuppressants, among others. Within 2–3 years, the entire immunological community jumped on the bandwagon, and since then, hardly an article has appeared in any immunological journal without mentioning the words T or B cells.

Over the years my work with colleagues has attracted prestigious awards (Box 2). The two I appreciate most are the Copley Medal from the Royal Society and the Prime Minister's Prize for Science. I treasure the former because it is the highest award granted by the Royal Society, and the oldest, the first medal being awarded in 1731. It is also a scientific award for outstanding achievements in any branch of science and previous medallists have included Charles Darwin, Francis Crick and Albert Einstein. I treasure the Prime Minister's Science Prize because it is a distinctly Australian Prize and is, at present, the highest award that is given here.

What does the future hold?

In 1971, Macfarlane Burnet stated, “None of my juniors seem to be worried as I am by the fact that the contribution of laboratory science to medicine has virtually come to an end”.¹⁰ Burnet would be greatly surprised and pleased by the technological breakthroughs,