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DISCOVERING THE ORIGINS OF IMMUNOLOGICAL COMPETENCE

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ABSTRACT

Work done in the late 1950s and in the 1960s revealed the role of the thymus in virus-induced leukemia in mice. Thymectomizing mice at birth to test whether the virus first multiplied in thymus tissue and then spread elsewhere ultimately led to the conclusion that the thymus was essential to the normal development of the immune system. Subsequent testing to try to understand how the thymus contributes to the pool of immunocompetent lymphocytes opened a new chapter in immunology and required a reappraisal of many immunological phenomena and an understanding of the molecular interactions that take place during cell-to-cell interactions.

Early Years and How I Ended Up in Australia

Both my father and mother were born in Paris in 1896. My paternal grandfather, Francis Meunier, was the headmaster of Lycée Henri IV in Paris, a learned man who had written books on the Greek and Latin languages. During the first World War, my father, Maurice Meunier, acted as interpreter of English for the British troops that came to France. In 1919, he married and left for China, having found a job in a French bank in Peking (now known as Beijing). He spent some 22 years in China and Japan, eventually becoming Manager of the Franco-Chinese Bank in Shanghai. Apart from English, he spoke Spanish fluently and learned Mandarin Chinese, which he could write, and also Japanese, which he wrote and spoke.

In 1930, my mother, who had followed my father to China, returned to France for health reasons. Finding that she was pregnant, she decided to have the baby in France, and so, having been conceived in China, I was born in France, in

Nice, in April 1931. In 1932, she went back to China with her three children, Jacqueline, the eldest, Jeanine, her second, and me, but she was back in France in 1935, both for her health and to allow Jacqueline to receive a “good” education at a boarding school. A year later, when we were just on the verge of returning to China, Jacqueline was diagnosed with pulmonary tuberculosis. Because of this, the family decided to go to Switzerland which, at that time, was the place where tuberculosis was supposed to be cured. We spent three years near Lausanne, and I do remember very well the doctor explaining to my mother what tuberculosis was and how little was known about the body’s resistance to such types of infections. In March 1939, my father joined us on a long service leave, but when the second world war broke out six months later, he was recalled to China. We hurriedly left Lausanne by car, crossing Northern Italy to Trieste, where we managed to get the last passenger boat out of Trieste.

When France capitulated, the French concession in Shanghai was taken over by Vichy officials, but my father, who did not accept France’s surrender, rallied to the Gaullists and became active politically. He smuggled young Frenchmen, who wanted to join the British forces, out of the French concession onto British ships leaving for Britain. In 1940, he was actually invited by the British War Office to join the London Headquarters as a link between the French and British Treasury. But in December of that year, only a few years before the discovery of streptomycin, Jacqueline died, aged 17. So my father finally declined the offer from London for the family’s sake, and especially because these were the months of the blitz. Nevertheless it was obvious that he had to leave Shanghai for he was next on the list of Gaullists to be arrested by the Vichy officials. He also knew that the Japanese would enter the war very soon and that he would be at great risk, as he spoke and wrote their language fluently. So some kind of deal was made with the British authorities in Shanghai: We were given British passports and our surname was translated into English—hence Miller. We left in August 1941, taking the last cargo boat out of Shanghai bound for Batavia (now known as Jakarta). There we boarded a passenger ship and arrived in Sydney on the 25th of September 1941, barely three months before the bombing of Pearl Harbor.

The Australians in Sydney did not recognize French banking credentials and thus would not employ my father on an equal footing. So he founded, together with another Frenchman, the Free French Delegation which took over the activities of the previous consulate, at that time defunct because Australia did not recognize Vichy. He offered his services to the Australian Government to translate any Japanese documents or information as required. He was also active in the war effort and helped with the taking in of supplies to New Caledonia.

Prior to arriving in Australia, Jeanine and I had never been to school. We had teachers at home, wherever we lived. The last one in Shanghai was a 36-year

old Viennese with a PhD who had escaped a Nazi prison. We had great fun with him and thought he must be very clever and wise to have a PhD at such a young age! Because my father had been impressed by the knowledge, culture, and broadmindedness of the Jesuits in Shanghai, with whom any subject could be discussed, he decided that I should go to a Jesuit college. He also thought that going to such a school would help me to get a better English accent and at least good manners! So I went to St Aloysius' College, and as there was a convent next to this, Jeanine went there. Although we did have a month's course in English before leaving Shanghai, we knew and understood so few words that we failed most of our exams during the first year, but we topped them all afterwards!

At St Aloysius I met and frequented a brilliant young Austrian boy (a refugee from Vienna) who was a year ahead of me (fortunately, because he also topped his class). His name was Gus Nossal and we became life-long friends. I have followed one year behind in his footsteps first at school, then at the Sydney Medical School, and finally at the Royal Prince Alfred Hospital in Sydney. Because I had witnessed Jacqueline's illness and had a great distaste for violence and war, I had wanted from an early age to study medicine and, if possible, to go into medical research. And so I was pleased to interrupt my medical studies for a year's research as a BScMed science student in the laboratory of Professor de Burgh, again following in the footsteps of Gus Nossal. I too was given the task of deciphering how ectromelia virus multiplied in liver cells, but rather than continuing on the line of work that previous BScMed students had performed with normal liver, I thought it more interesting to determine whether the virus might interfere with some crucial biochemical events during liver regeneration after partial hepatectomy. Two papers resulted from this work (1, 2).

After receiving my medical degree and doing an internship in Sydney, I applied for what was called a Gaggin Research Fellowship, advertised in the *Medical Journal of Australia*. It was given by the University of Queensland, Brisbane, and offered a return fare to the United Kingdom and a salary for two years in a Research Institute of the candidate's own choosing. I was lucky to get this Fellowship, and I applied to many Institutes in England. Most were unable to take me, but one, the Chester Beatty Research Institute, an Institute of Cancer Research, in South Kensington, London, accepted me as a postdoctoral student for the PhD degree of the University of London.

I arrived in the United Kingdom in 1958, not knowing exactly what I was going to do. Many of the scientists at the Institute were heavily involved in searching for new chemical carcinogenic compounds. Adding more compounds to an evergrowing list of carcinogenic agents did not interest me, as I would rather have used the experience I gained in my B.Med.Sci. year to work on some model in which pathogenetic mechanisms had to be investigated. Hence, I felt

rather frustrated and did not really want to work in any of those laboratories. I was then told that the Institute had two satellites outside greater London, one being at a place called Pollards Wood, at Chalfont St Giles in Buckinghamshire. There, Dr. RJC Harris was working on the development of sarcomas in turkeys caused by the Rous sarcoma virus, and this line of investigation I thought might perhaps interest me. So I visited him. Instead of joining his group and working on some aspects of the Rous virus, he suggested that I might be willing to investigate the pathogenesis of lymphocytic leukemia induced in mice by what was presumed to be a virus that had recently been discovered by Ludwik Gross in the United States (3). This suited me perfectly. As a PhD student I was under the supervision of Dr. Harris, although my official supervisor had to be a full Professor of the University of London, which in my case was Professor Sir Alexander Haddow, the director of the Chester Beatty Research Institute.

Pollards Wood was a large estate that had previously belonged to Bertram Mills, the circus owner. It had a magnificent Tudor-style mansion sitting in the middle of beautiful gardens and woods. The rooms had been refurbished as well-equipped laboratories and offices. There was also a kitchen and a dining room. All the buildings scattered throughout the estate that had previously housed animals such as horses, dogs, and elephants had also been converted to laboratories or animal quarters. A van from the main Institute in South Kensington came every day to bring mail and whatever supplies were required. Even though I was given only a small amount of space in one of the converted horse stables, it was a delight to work in such pleasant surroundings, away from the crowd, the noise, and the pollution of greater London.

The Thymus in Mouse Leukemia

In the late 1950s, many scientists, including Haddow, did not believe that cancer in mammals could be caused by viruses. Gross had in fact not isolated a virus as such, but had been able to induce leukemia in so-called “low-leukemia” strains of mice, such as C3H, that normally did not develop the disease. He had done this by simply inoculating newborn mice with filtered extracts of leukemic tissues from “high-leukemia” strain mice, such as Ak [3]. Furthermore, not all low-leukemia strains developed the disease after such inoculation, e.g. C57BL mice were highly resistant, and even C3H mice in some laboratories were not as susceptible as mice of Gross’s own C3Hf/Gs strain. Repeating Gross’s observations using the strains of mice available at Pollards Wood might have taken months or years, and so I decided to write to Gross asking whether he would be kind enough to send his virus or mice harboring it. To my great relief, Gross accepted.

My first experiment was just to repeat Gross’s original findings using his own C3H strain, and I soon confirmed the results he had described. It was

known at that time that lymphocytic leukemia in mice involved the thymus and that adult thymectomy prevented spontaneous leukemia developing in high leukemic strain mice. It also prevented leukemia in low-leukemic strain mice otherwise induced by ionizing radiation and chemical carcinogens. As no one had yet investigated the role of the thymus in virus-induced leukemia, I thought this would be a good starting point for my PhD studies. Many questions had to be answered. Could thymectomy impair the leukemogenic process in virus-inoculated mice? Could the virus multiply anywhere or only in thymus tissue? What would implantation of a normal syngeneic thymus achieve in mice whose own thymus had been surgically removed? Would thymectomized mice from high leukemic strains implanted with thymus tissue from mice of low leukemic strains develop leukemia? These questions seemed to me to be relevant, and I immediately set out to investigate them. But to do so, I required large numbers of mice of different inbred strains and hence considerable animal space that was not available. Six months after my arrival, however, Harris was offered the directorship of the Division of Virology of the Imperial Cancer Research Funds at Mill Hill, London. I was therefore left without an immediate supervisor, but I was really very fortunate to acquire his animal space and a small shack.

I inoculated C3Hf/Gs mice with leukemic extracts immediately after birth and thymectomized these mice about 4 to 5 weeks later. None developed leukemia (4). Implantation, in such thymectomized inoculated mice of neonatal thymus tissue taken from uninoculated syngeneic mice restored the potential for leukemia development (5). A similar effect of thymectomy and thymus grafting had previously been observed in high leukemic strain mice or in low leukemic strain mice given irradiation or chemical carcinogenic agents (6). What would happen, however, if a high leukemic strain mouse (Ak) were thymectomized and implanted with thymus tissue from a low leukemic strain donor (C3H)? Since donors and hosts were allogeneic, this could be studied only in mice immunologically tolerant to the donor tissues. Although this aspect of my unsupervised work now appears quite naive, it did pave the way for me to perform experiments with immunologically tolerant mice, the phenomenon of tolerance having fascinated me since 1953 when, as a medical student, I had read in *Nature* the first report of its existence by Medawar and collaborators (7). Needing to learn how to inject newborn mice intravenously and how to skin graft to check whether tolerance had been established, I had therefore an excuse to approach Sir Peter Medawar and his group. An opportunity arose when Medawar delivered the Tercentenary Lecture of the Royal Society in London. It was an inspiring and stimulating talk given with clarity and wit. I learned how foreign tissue grafts were rejected and how tolerance to these might be induced experimentally by the inoculation of foreign hemopoietic cells into embryos or newborn animals. I

approached Medawar with my problem, and he kindly asked his collaborator Leslie Brent to show me the techniques of intravenous injections into newborn mice and skin grafting. Leslie was a very nice teacher, and I am most grateful to him for spending so much time with me in those early days. Medawar, knowing that I was thymectomizing adult mice, said jokingly: "now that we have shown you how to inject newborn mice intravenously, perhaps one day you will show us how to thymectomize newborn mice." What a prophetic statement!

The results I obtained in immunologically tolerant Ak and C3H mice were clear-cut: Neoplasms developed in Ak thymuses grafted to thymectomized C3H mice. Some of the tumors arising in the thymus graft regressed after injection of lymphoid cells from C3H mice immunized against Ak tissues (8). Although I was immensely pleased with these results, they were not breaking new ground, being generally in accordance with previous work on leukemogenesis and on the induction and breaking of immunological tolerance. But the experience I gained in all this work was invaluable.

Effects of Neonatal Thymectomy

As mentioned above, for leukemia to develop in those early days, the leukemic extracts had to be given at birth. Furthermore, inoculated C3Hf/Gs mice failed to develop the neoplasm when thymectomized after weaning but did so when subsequently grafted with syngeneic thymus tissue. What was most fascinating was the finding that grafting the thymus *as late as 6 months* after thymectomy still allowed leukemic transformation (5). The virus must clearly have remained latent, and it was indeed recoverable from the healthy nonleukemic tissues of neonatally inoculated thymectomized mice (9). Why should the virus be given at birth and not later? Where could it have multiplied? One possibility that I entertained, was that leukemogenic transformation occurred only if the virus could first multiply in the developing thymus. Thymectomy performed at weaning would remove the source of the malignant cells but not the virus, which would have spread to other sites and which would thus be available to transform cells whenever a neonatal thymus was grafted. If this were true, neonatal mice lacking a thymus from birth should no longer be susceptible to virus infection and would not develop leukemia when later grafted with thymus tissue. To test such a hypothesis, I had of course to teach myself the technique of neonatal thymectomy. After numerous attempts, I finally worked it out and thus had little immediate surgical mortality. Cannibalism by the mothers was, however, a major problem, and so I had to thymectomize large numbers of baby mice. I am most grateful to my wife Margaret who, although working as a technician in the Drosophila Laboratory at Pollards Wood, gave up much of her spare time at nights and weekends helping me thymectomize the babies and coaxing their mothers into not eating them! The survivors grew well at

first but, after weaning, many wasted 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 or obvious pathology. This led me to conclude “that the thymus at birth may be essential to life” (10).

Histological examination of the tissues of neonatally thymectomized mice revealed a marked deficiency of lymphocytes in the circulation and the lymphoid tissues, and many wasted mice had liver lesions suggesting infection by some hepatitis virus. Perhaps I might not have followed up these findings, had I not heard of the brilliant work of the famous immunologist Jim Gowans. He had recently shown that small lymphocytes were not short-lived cells, as had been thought before. On the contrary, they were immunologically competent cells with a long lifespan, recirculating from blood through lymphoid tissues into lymph and able to initiate immunological reactions when appropriately stimulated by antigen (11). Clearly, my neonatally thymectomized mice must have been immunodeficient, which accounted for their susceptibility to virus infections. I therefore tested their immune competence by grafting skin from allogeneic mice and from rats. The results were incredibly spectacular. The mice failed to reject such skin and failed to do so even when grafted *before* the onset of wasting. The grafts grew luxuriant tufts of hair and, to convince myself, I even transplanted some mice with four grafts, each from a different strain with a different color, some strains differing from the recipients at the strong histocompatibility locus, H-2. None of the grafts were rejected, and the recipients looked like having a patchwork quilt on their back! Since both Gowans and Medawar had firmly established that rejection of foreign skin grafts was mediated by lymphocytes, and since my mice were deficient in lymphocytes following *neonatal* thymectomy, it was logical for me to postulate that the thymus was the source of immunologically competent lymphocytes, at least during the neonatal period.

At that time, a thymus immune function was unlikely to be accepted by the immunological community. There were many reasons for this. Unlike small lymphocytes taken by thoracic duct cannulation and unlike spleen and lymph node cells, thymus lymphocytes were generally poor in their ability to initiate immune reactions after adoptive transfer to appropriate recipients. Thoracic duct lymphocytes could home from blood into lymphoid tissues, “the only exception” being “the thymus in which very few small lymphocytes” appeared “to lodge” (11). The production of antibody-forming plasma cells and the formation of germinal centers, so prominent in spleen and lymph nodes, were not seen in normal thymus tissue. Defects in immune responsiveness had never been documented in mice whose thymus had been removed during adult life, a fact that had led some groups to conclude that “the thymus gland does not participate in the control of the immune response” (12). At a symposium

on *Cellular Aspects of Immunity* (13), published in 1960, in which world-renowned immunologists, including Burnet, Good, Lederberg, Medawar, and Mitchison, took part, not a single reference was made to the thymus or to its cells. Immunologists believed that, as a predominantly epithelial organ, the thymus had become vestigial during evolution and was just a graveyard for dying lymphocytes. Even in literary circles, the thymus seemed to have influenced writers with strange ideas. Lawrence Durrell in the *Alexandria Quartet*, for example, spoke of “the satiny skin that is given only to the thymus-dominated” (14), and as recently as 1971, one medical dictionary stated: “The function of the thymus gland is still obscure. One theory concerning its function is that it is concerned with general sexual maturity” (15).

Faced with so much evidence against an immunological function for the thymus, I hesitated to publish my results immediately, but wished to receive some feedback from well-known immunologists. A summary was sent to some of them and I also spoke about my findings at various meetings in London, Oxford, and Perugia. When slides of my neonatally thymectomized mice bearing four different skin grafts, looking like a patchwork quilt, were shown at the British Society for Immunology Meeting in 1961, people were stunned, but my conclusions were regarded with skepticism. For example, Medawar was not convinced as was evident from a letter to me in which he wrote: “I take it that the thymic tissue seen in fishes is wholly or predominantly epithelial, as its phylogenetic origin suggests. It is a matter of some interest that many organs which seem to become redundant in the course of evolution undergo a sort of lymphocytic transformation” (16). Trivial criticisms abounded: What I had observed must surely have occurred only in the strain of mice that I had been using; my mice must have been in such poor health that any surgical trauma would prejudice their ability to reject skin grafts; whatever the thymus might have been doing in my mice, it could not possibly do in humans! At a Ciba Foundation Symposium on *Tumour Viruses of Murine Origin* held in Perugia in June 1961, the first *international* meeting where I presented results, my former mentor, RJC Harris, claimed the following: “Dr. Delphine Parrott in our laboratory has been thymectomizing day-old mice and there is at present no evidence that these animals are immunologically weaker than normal animals. They do not retain skin grafts, they are living and breeding quite normally. They do not die of laboratory infections” (17). All these comments and criticisms worried me a lot, and I decided again to repeat my work on even larger numbers of mice of different strains. But at that time, Sir Alexander Hadow, who had also attended the Perugia Meeting, urged me to immediately submit my initial results for publication. He suggested the medical journal, *The Lancet*, as I had already five papers in *Nature* and that journal might not have accepted a paper on such a controversial topic. I therefore sent a brief report to *The Lancet*

and, contrary to the prevailing opinion, I postulated 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 lymphocytes leaving the thymus are specially selected cells” (18). I had therefore proposed the bold postulate that the thymus was the site responsible for the development of immunologically competent small lymphocytes. This was the very first publication showing data supporting the immunological function of the thymus.

Soon after this, I sent an application to present a paper at the New York Academy of Sciences meeting which was to be held in February 1962. This was accepted. It was my very first visit to the United States, in the middle of a harsh winter the likes of which I had never experienced. I gave my results in great detail, emphasizing that mice thymectomized at birth failed to reject skin both from totally unrelated strains (“H-2-incompatible”) and from other species such as rats (19). In the ensuing discussion, Martinez from Good’s group bluntly stated, without providing any data, that they also had shown that neonatally thymectomized mice were somewhat immunodeficient but, in contrast to my findings, prolonged skin graft survival occurred only in mice identical at the H-2 histocompatibility locus but differing at other weaker histocompatibility genes. Their mice did reject skin from H-2 incompatible strains. It seems strange that this group who later claimed to have had such results in 1961 (20), gave at this New York Meeting, in February 1962, a paper which was not on the thymus and in which the word thymus did not appear (21). They did, however, publish their findings later in 1962, again emphasizing the ability of their neonatally thymectomized mice to reject H-2-incompatible grafts (22). Such a discrepancy between their results and mine was later explained by their admission that they had not completely thymectomized their mice: “Careful autopsies performed in the thymectomized animals often revealed minute amounts of residual thymic tissue in these animals. With perfection of our technique a large proportion of neonatally thymectomized mice accepted H-2 incompatible grafts in contrast to partially thymectomized mice” (23).

At the end of 1961, I had accumulated a large amount of data on the effects of thymectomy in newborn mice and on their rescue by normal syngeneic lymphocytes or by implanting thymus grafts. Although implantation of syngeneic thymus tissue allowed these mice to develop a normal immune system, grafting a thymus derived from a foreign strain induced specific immune tolerance to the histocompatibility antigens of the donor. Thus, lymphocytes developing in the thymus in the presence of foreign cells must have been deleted [i.e. “selectively thymectomized” as I suggested (24)]. Hence, by implication, the thymus should be the site where self-tolerance is imposed and where discrimination between self and nonself takes place. Sir Alexander Hadow again urged me

to send all these detailed results for publication, and he kindly communicated them on my behalf to the *Proceedings of the Royal Society Series B* in late December 1961 [received by the *Journal* on January 5, 1962, and published later that year (24)]. I was also invited to present my work at the Royal Society in May 1962. My suggestion that the thymus could be involved in tolerance induction received strong support from Sir Macfarlane Burnet who stated in a lecture given at the University of London in June 1962: "If, as I believe, the thymus is the site where proliferation and differentiation of lymphocytes into clones with definable immunological functions occurs, we must also endow it with another function—the elimination or inhibition of self-reactive clones" (25). Burnet, having read the results I had obtained with neonatally thymectomized mice, was in fact one of the rare immunologists who believed in an immunological function of the thymus. It was during his 1962 visit to London that I had my first chance to speak to him.

In 1963, I was awarded an Eleanor Roosevelt International Fellowship that allowed me to work for one year at the National Institutes of Health in Bethesda, in Dr. Lloyd Law's department. There I neonatally thymectomized germfree mice and proved that these remained healthy after weaning, but yet were still unable to reject foreign skin grafts (26). With Law and collaborators, I consolidated my earlier observations that mice lacking a thymus were much more prone to develop neoplasms (27), thus adding weight to Burnet's hypothesis of immunological surveillance.

As mentioned before, Good's group in the United States, working independently from me, soon confirmed the results I had already published. Furthermore, another group led by Waksman also obtained in rats similar results, which appeared in the scientific literature in 1962 (28).

The Thymus in the Adult

In adult mice, thymectomy had for long been known not to produce any immune defects (29). Since total body irradiation destroyed lymphoid tissues, I reasoned that recovery of immune function following irradiation might be thymus-dependent. Mice were thymectomized after weaning and subjected to a sublethal dose of total irradiation. Whereas sham-thymectomized controls eventually fully recovered immune functions, the thymectomized mice remained immunocompromised. These results were sent to *Nature* and published in 1962 (30).

At that time, a group at the Chester Beatty Research Institute in South Kensington, headed by Professor Koller, was studying the effects of heavy doses of irradiation on the hemopoietic system and its regeneration following an intravenous injection of bone marrow cells. Having just demonstrated the importance of the adult thymus in the recovery of immune function after

irradiation, I approached Koller's co-workers and persuaded them to collaborate with me to test the hypothesis that, in "lethally" irradiated mice lacking a thymus, only the hemopoietic tissues but not the lymphoid tissues would regenerate after bone marrow injection. A very close collaboration thus ensued between Pollards Wood and the main Institute, and the results were exactly as predicted (31, 32). Since then, the technique of adult thymectomy, irradiation, and marrow protection has been used continuously for numerous experiments in cellular immunology.

Two Major Lymphocyte Subsets

The work of Gowans, in the early 1960s, had shown that the recirculating small lymphocytes, in mammalian species, appeared to belong to a homogeneous population able to give rise to cells involved in both cellular and humoral immunity (11, 33). There was no reason to believe in the existence of separate subsets. If this were so, must all lymphocytes be thymus-derived? Of course neonatally thymectomized mice still had some lymphocytes, but these might have migrated from the thymus prior to birth. In birds, however, preventing the development of the other thymus-like organ, the bursa of Fabricius, by testosterone injection was known since 1956 to be associated with defects in antibody production in the mature bird (34). Burnet and his colleagues repeated and extended this work; they documented a division of labor among chicken lymphocytes, early bursectomy being associated with defects in antibody formation and early thymectomy with defects in some cellular immune responses (35). Since I had shown, however, that neonatal thymectomy in mice prevented *both* cellular and humoral immune responses (24, 36), Burnet was led to conclude that in "mammals it is highly probable that the thymus also carries out the function performed by the bursa of Fabricius in the chicken, which is to feed into the body the cells whose descendants will produce antibody" (37). But then, why did neonatally thymectomized mice show a deficiency of lymphocytes limited to those areas of lymph nodes and spleen known to be associated with changes induced by cellular immune responses, but not those areas where antibody-producing cells appeared (38)?

A clue to this mystery came from a totally different line of investigation. Claman and his collaborators in Denver showed that irradiated mice receiving a mixture of marrow and thymus cells produced more antibody than controls given either cell source alone (39). As their model lacked genetic markers, it could not determine the origin of the antibody-forming cells. Thus the function of bone marrow cells might simply have been to protect the irradiated mice, thus allowing cells in the thymus inoculum to produce antibody. Davies and his collaborators, in Koller's department, attempted to follow up this work by using adult thymectomized irradiated mice given bone marrow and thymus

grafts from donors that had slight immunogenetic differences (40). These chimeras were challenged with sheep erythrocytes and their spleen cells transferred into irradiated recipients presensitized against either the thymus or the marrow donor. Those able to reject cells with the immunogenetic markers of the thymus donor produced antibody. Those immunized against the marrow donor produced much less. Since these transfer experiments were performed 30 days after irradiation and thymus grafting, at a time when the lymphoid cell population of the thymus graft had been entirely replaced by cells of marrow origin, as I had previously shown (41), the results were difficult to interpret. Thus hemolysins in the irradiated recipients presensitized against the thymus donor might well have been produced by marrow-derived cells that had first repopulated and then emigrated from the thymus graft. The antibody-producing cells would then have had the immunogenetic characteristics of the marrow donor and yet be thymus derived. Davies himself concluded: "It may be that thymus-derived cells can produce antibody, but only in the presence of cells of bone marrow origin. Equally cells of bone marrow origin may be the cells whose immunological potential is enhanced by association with cells of thymic origin. These are not problems which the present analysis can resolve" (42).

In 1965, I was invited back to Australia by Professor Gustav 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 for me, as my first PhD student, a brilliant young man, Graham Mitchell, who had just graduated with first class honors from the University of Sydney Veterinary School. Graham was a delightful person to work with, and we too became life-long friends. We wanted to understand how the thymus contributed to the pool of immunocompetent recirculating small lymphocytes, and to achieve this we first investigated the ability of various cell types to restore immune functions in thymectomized mice. We inoculated cells from F1 hybrid mice into neonatally thymectomized or thymectomized-irradiated recipients of parental genotype, so as to have genetic markers. Since thymus cells were poor at initiating antibody responses, we used thoracic duct lymphocytes or thymus cells that had twice been serially transferred with antigen into two sets of irradiated mice (we even named these "educated thymus cells"). Bone marrow cells, "educated" thymus cells, or thoracic duct cells, when given alone to irradiated mice, produced only little or no antibody in response to sheep erythrocytes, as measured by a plaque technique on erythrocyte coated agar plates (each plaque representing a single antibody-forming cell). When, however, the "educated" thymus cells or thoracic duct cells were injected together with bone marrow cells, the plates were crowded with plaques. By using anti-H-2 sera, we were now able to determine which cell type produced the antibody. While the plates were incubating with specific

antibodies able to eliminate either thymus-derived or bone-marrow derived cells, we waited anxiously for the results. My bet was that the antibody-forming cells would be thymus derived, but I have always bet the wrong horse! The results could not have been more spectacular: One set of plates had just nothing on it, the other was as crowded with plaques as before. Now, which set was which? We decoded the experiment and, of course, I lost my bet. But I was elated by results so convincing and so exciting that I felt just as “the lark at break of day arising from sullen earth, sings hymns at Heaven’s gate” (43)! Our work established, definitely, unequivocally, and for the *first* time, the existence, in species other than birds, of two major subsets of lymphocytes: antibody-forming cell precursors derived from lymphocytes in bone marrow, and thymus-derived cells essential to allow the former to respond to antigen by producing antibody. We sent a note to *Nature* (44) and a polished paper to the *Proceedings of the National Academy of Science of the United States of America* (45). The latter was unfortunately delayed by the 1967 Christmas mail. We of course performed a great deal of work that gave results as convincing as the early ones, and we proved beyond doubt that some interaction took place between these two major subsets of lymphocytes in antibody responses. We sent four papers to the *Journal of Experimental Medicine*, and these were accepted and published back to back (46–49). As a light exercise, I tried to find acronyms for the clumsy words thymus-derived, bone marrow-derived or antibody-forming cells precursor, but nothing pleased me. It was left to Ivan Roitt, the immunologist responsible for the world’s most popular textbook, to coin several years later the simple and minimalist terms, T and B cells (50)!

How did the immunological community react to our findings? There was complete surprise, of course, but there was also disbelief when I presented these results at meetings held in the United States and Canada in 1968. At Val Morin, 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 cells would never find each other. At a meeting in Brook Lodge, held in 1968, Gowans, who had clearly shown that recirculating small lymphocytes could initiate *both* cellular and humoral immune responses, stated: “Had it not been for Dr. Miller’s experiments I would have assumed that a single variety of small lymphocyte was involved in each of our experiments” (51). At the same meeting, Good was “concerned at separating thymus-derived from marrow-derived cells” since the former “are in fact, marrow-derived cells” (52). Even Burnet, despite his own work with chickens, expressed doubts “about the significance of results obtained in such biological monstrosities as pure line mice thymectomized, lethally irradiated, and salvaged by injection of bone marrow from another mouse” (53). The most sarcastic criticism came from Professor Bede Morris, the then Professor of Immunology at the John Curtin School of Medical

Research in Canberra, Australia, who likened B and T cells to the first and last letters of the word “bullshit”.

In spite of all these criticisms, Graham and I persevered in our work. It was urgent to re-examine a multitude of immunological phenomena in terms of the two cell system: tolerance, memory, the carrier effect, autoimmunity, immune deficiency, genetically determined unresponsive states, original antigenic sin, etc. Within two to three years, the entire immunological community jumped on the band wagon, and since then, hardly an article has appeared in any immunological journal without mentioning the words T cells or B cells.

Conclusions

Younger investigators working in cellular immunology are probably quite surprised at the account I have given. For today, the immunological function of the thymus is taken for granted, as if it had never ever been in doubt, and T and B cells have become household words. Research has progressed so fast in the last three decades that we can now probe the molecular basis of the interactions between T cells and their ligands and between T cells and other cells such as B cells and dendritic cells. Yet we have a long way to go, and after 40 years in medical research, I am still keen to find out exactly how lymphocyte homeostasis is maintained (e.g. 54), how T cells, B cells, and other cells interact (e.g. 55), and why the immune system fails to respond to self under normal conditions (e.g. 56, 57).

In 1971 Burnet (58) 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.” A similar outlook on the future of surgery was held in 1930 by the famous surgeon Lord Moynihan (59): “We can surely never hope to see the craft of surgery made much more perfect than it is today. We are at the end of a chapter.” Yet would not both Burnet and Moynihan be greatly surprised and pleased by the technological breakthroughs and novel experimental approaches that have given us so much new knowledge both in surgery and immunology? And although we can employ numerous strategies to allow better survival of transplants, to deal with various forms of immunological aberrations, and to produce new vaccines, we still have to learn a great deal, in particular how to apply clinically the fundamental knowledge obtained from our bench work. I am thus in full agreement with the late Sir 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” (60). As Sir Winston Churchill once said: “So much accomplished, so much still to be done.”

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