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Gordon Leslie Ada 1922–2012

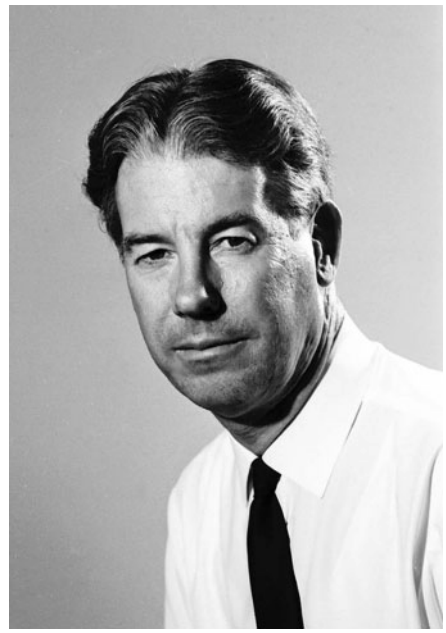
Gustav J. V. Nossal[A,C] *and Christopher R. Parish*[B]

[A]Department of Pathology, University of Melbourne, Vic. 3010, Australia.
[B]John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia.
[C]Corresponding author. Email: gnossal@bigpond.net.au

Gordon Ada, an outstanding virologist and immunologist, was the first to demonstrate that the influenza virus genome is composed of RNA, not DNA. In immunology he provided evidence that refuted the template theory of antibody formation and performed elegant experiments to prove Burnet's Clonal Selection Theory. His administrative skills created a research environment that nurtured the Doherty-Zinkernagel Nobel Prize in viral immunology and allowed him to assist WHO and others greatly in developing effective vaccines.

Early Years, Education and Family Life

Gordon Leslie Ada was born on 6 December 1922, in the Sydney suburb of Drummoyne, the fourth of six children. His father, William Leslie Ada, was educated at Fort Street and Sydney Boys High Schools and studied engineering at the University of Sydney, where he excelled academically and at rowing. William Ada joined the staff of the New South Wales Department of Railways where he spent his entire career, rising to Chief Electrical Engineer. He married Erica Maud Flower, a second cousin, the daughter of a builder. Erica had lost her mother at the age of nine and received a minimum of schooling. Gordon Ada described his childhood as happy, dominated by his very kind father. Both parents were religious and observant Presbyterians. It appears his father was rather introverted, entertained very little and, because of the depression, lived economically and austerely. Gordon attended local state schools for all but his last two years, recalling Mr Philpot who taught music, awakening a life-long interest, and Mr Monahan who gave him a good grounding in English, Latin and mathematics. Moving to Fort Street Boys High School for 1938 and 1939, Gordon studied for his Leaving Certificate where he excelled in history and science.



While studying medicine was considered, a book by H. G. Wells, Julian S. Huxley and G. P. Wells entitled *The Science of Life*, had triggered an interest in the chemistry and physics of living things, so in 1940 Ada started a science degree at the University of Sydney. Fondly remembered were Professor Eric Ashby and Dr Rutherford N. Robertson in botany and Dr F. Lions in organic chemistry. Sir Rutherford Robertson was to become a distinguished President of the Australian Academy of Science. Dr Jack L. Still was a strong influence in biochemistry, as a result of which Ada did a fourth (honours) year in this subject. As we shall see, a research topic picked up during this year, 1943, was to have a profound effect on Ada's later career, but before describing

Figure 1. Unit Heads at the Walter and Eliza Hall Institute in 1966: Gordon Ada, Ian Mackay, Gus Nossal (Director), Don Metcalf and Jacques Miller, now all FAAs (photo provided by the authors).

the relevant research, some further reflections on Ada as a person may be apposite as both the authors knew him well (Fig. 1).

Ada met Jean Macpherson in 1944 and they married in 1946. The daughter of an architect, Jean was educated at Melbourne Girls' High School, and joined the staff of the Commonwealth Serum Laboratories where she worked until her marriage. Four children were born, Ian Douglas (1947), Andrew Leslie (1951), Louise Margaret (1954) and Neil Ross (1956). Ian is an agricultural scientist, working for over 30 years in the Victorian Department of Agriculture, but now in economic development in local government. Andrew is a general medical practitioner in Melbourne, Louise is Professor of Physiotherapy at Sydney University, and Neil has a PhD in forestry, but now works in the Australian Maritime Safety Authority. Ada has four grandsons and twin great-grandsons.

Ada's children remember their father when they were young as either working or spending time with the family, gardening, landscaping, or being a home handyman. He would often go to work at the Walter and Eliza Hall Institute on Saturday mornings and, as the children got older, he would take them with him, where they would spend time sliding down the bannisters in the stairwell or helping Dr Margaret Holmes in the Animal House. The younger children remember before they were ten years old becoming very competent at removing the digestive tracts of rats. Ada never used the lifts at the Institute. Because he did not play sport, climbing the stairs whenever he could was his fitness program. The family would then go to the Queen Victoria Market to buy the weekly fruit, and vegetables that they did not grow themselves. The family had a traditional quarter acre block in what, in the 1950s, was an outer suburb of Melbourne, so there were fruit trees, and a vegetable garden as well as plenty of space for lawn. Ada conveniently left room to allow for a full-length cricket pitch, so many lazy hours in summer were spent playing cricket, or splashing in a canvas wading pool that he constructed.

Another memory of the children is of Ada writing at his desk in the evenings, presumably on drafts of scientific papers. He also wrote to his father and mother in Sydney every weekend at a time when telephone calls were very expensive. A benefit to the children of their father attending overseas conferences was that he would bring back components for a Triang electric train set that were much cheaper to buy in England, and in later years, Beatles' LP records before they were released in Australia. Corresponding with scientists all over the world meant most of the children had wonderful stamp collections.

With his interest in the outdoors as a young man, Ada encouraged his children to join Scouts or Guides. For some it has led to a lifelong interest in outdoor activities. He encouraged all the children to do the best they could at school, but was always supportive and never demanding—he was clearly a strong influence as all the children did science-based degrees. Their mother's love of art and literature did not figure in any of their formal studies, although Louise has used her creative talents to help friends with room design and layout for renovations for many years. Ada loved classical music and he often 'air' conducted performances played on his (initially) monophonic gramophone. He eschewed popular music but grudgingly admitted that the Beatles were slightly melodic at times. His favourite music was from the seventeenth to nineteenth centuries—the great symphonies and, particularly, the piano and violin concertos. He encouraged the children to learn music, and Andrew matriculated in music with the clarinet.

Ada working hard was not without its benefits for the children, with the year spent in London in 1964 being a highlight of their family life. Other highlights were the to-and-fro on P&O through the Suez and Panama Canals, and a wonderful summer holiday in Switzerland and Austria, although sometimes there was a little rebellion at the number of castles, ruined abbeys and stately homes the family visited on weekend drives.

As a scientist with a growing reputation, Ada was very modest and unassuming with his family. He did not discuss his work in any detail, but was always happy to debate the issues of the day at home. With the rapidly changing cultural mores of the late 1960s and early 1970s these topics included the Vietnam War, drugs and sex before marriage.

Ada had loved sailing on Sydney Harbour as a teenager and young man, with the garden of the family home running right down to the Parramatta River at Drummoyne. This interest was rekindled when the family shifted to Canberra and he sailed a Northbridge Senior on Lake Burley Griffin, with either Louise or Neil as crew. They report that he had a version of sportsman's 'white line fever' when he sailed, as it was the only time they ever heard him swear. Once all the children left home he sailed a Laser for several years.

Ada took a great interest in his grandchildren once they came along. They remember him reciting stories and poems, and 'Peter and the Wolf' was a favourite. He loved them helping in his large garden, particularly harvesting vegetables, and he would explain his scientific approach to composting. Another main task was for him to adjudicate at cricket matches on the back lawn. Also, the grandchildren received postcards, and matchboxes from hotels, from the many weird and wonderful places Ada visited. This exposed them to the world and developed their love of travel. When they grew older he was always ready and chuffed to be a source of advice on university choices and career moves. They also remember their grandfather saying that having a family was his greatest accomplishment. A wonderful example of love and commitment to family was demonstrated to them by Gordon taking their grandmother tea and toast in bed every morning, and his devotion to her care in the last long years of her illness.

Jean and Gordon's happy marriage was to become overshadowed by Jean's severe and chronic illness that eventually required institutional care. Gordon remained the most faithful carer. Jean died in 2005. Eventually he, too, felt the ravages of ill health. Pathology in the vertebral column caused pressure on his spinal cord resulting initially in foot drop and an impaired gait, and later the adoption of a very bent posture. Towards the end, he was more comfortable in a wheelchair. Nevertheless, he participated fully in scientific events, for example a symposium in 2011 to mark the 80th and 90th birthdays of four former Walter and Eliza Hall Institute colleagues. His declining mobility finally forced him to move into care where he lived happily for four years before dying peacefully in Canberra

from the 'gentlemen's friend' on 25 September 2012.

Scientific Career and Research Accomplishments

As a scientist, Ada was particularly thorough and meticulous. He had a fondness for the techniques of biochemistry and biophysics. In his research, he had impeccably high standards of scientific rigour and integrity. In his scientific interactions, he was gentle and gentlemanly, but at the same time he could be quite strong and forceful, especially when he was defending a scientific position. As a scientific colleague, he was constructively critical and also immensely loyal, in fact a joy to work with. As a lecturer, he was methodical and informed, and as a teacher, patient and insightful.

One of us (CRP) was fortunate enough to experience Ada's approach to science and his supervisory style first hand, being a PhD student in his laboratory at the Hall Institute from 1966 to 1968. Consistent with his high standards, right from the start Gordon insisted on a meticulous approach to experiment design and the rigorous interpretation of any data generated. He also encouraged students to be prepared to spend a considerable amount of time perfecting experimental procedures as 'any data obtained is only as good as the assay used to generate it.' Combined with this rigorous approach to experimentation Gordon encouraged his students to be always prepared for the unexpected experimental result. His supervisory style was relaxed. He was always keen to discuss science and preferred to hear about the results as they occurred rather than waiting for scheduled meetings. He also encouraged his students to pursue their own ideas, although he often acted as a polite but firm devil's advocate when new ideas were discussed. Most importantly, he was very supportive of students when the progress of their research faltered and adept at providing innovative solutions. Based on these attributes, it is no surprise that many of Ada's PhD students subsequently had very successful research careers.

Ada was a highly productive scientist. Over a sixty-year period, from 1948 to 2008, he published 318 scientific publications, including three co-authored books, two edited books and over 70 book chapters. A complete bibliography of all of his publications is available online as Supplementary Material. As would be expected of such a productive scientist, during his research career Ada made several important contributions to science, particularly in the fields of virology and immunology. Some of his most significant research accomplishments are highlighted below.

Serum Fractionation

During his Honours year in 1943, Ada came under the influence of Major Robert J. Walsh. Bob Walsh was a remarkable person and a major academic power broker who was at that time in charge of the Red Cross Blood Transfusion Service. It appeared that human serum became turbid on storage and finally developed a precipitate rendering its transfusion rather dubious. Walsh asked the Department of Biochemistry to investigate this and the task was assigned to Ada. Pre-treatment with organic solvents helped and Ada was funded to continue the work in 1944. Major 'Val' Bazeley, of penicillin and Salk vaccine fame, invited Ada to the Commonwealth Serum Laboratories in Melbourne where a large batch of serum was successfully processed. Unfortunately for Ada, an essentially similar ether extraction process was published from the Lister Institute in London. Shortly after, E. J. Cohn and a large team from Harvard University came up with a definitive alcohol fractionation procedure for serum that soon became the industry norm. Ada was invited to join the Commonwealth Serum Laboratories' staff and, although a commercial process was not immediately established, enough good work was done to write a thesis on 'The Behaviour of Blood Serum during Storage' that was accepted by the University of Sydney for a Master of Science Degree.

Ada soon realized that the equipment, techniques and expertise for a penetrating study of serum proteins simple did not exist in Australia. If he were to progress in this field, overseas experience was essential. He wrote to Dr A. S. Macfarlane at the National Institute of Medical Research in London and was accepted in principle. However, he failed to get funding support from the Commonwealth Serum Laboratories and so, intrepidly, resigned and set off anyway for London in 1946. Within three months he gained

a position and began work on electrophoretic investigation of serum. Some year and a half later in mid-1948, Ada accepted an offer from Professor F. M. (later Sir Macfarlane) Burnet to set up a biophysics unit at The Walter and Eliza Hall Institute of Medical Research in Melbourne, then commonly known as the Hall Institute. Together with Mr Henry F. Holden, much of the equipment, including electrophoresis apparatus and an ultracentrifuge were designed and constructed in-house.

Virus-Cell Interactions and the Receptor Destroying Enzyme

In the late 1940s and early 1950s much of the Hall Institute's work was directed towards understanding basic mechanisms of virus replication. To attach to a cell, surface molecules of the virion had to react with cell surface receptors. Later, to infect other cells, virions had to detach from the infected cell. Burnet was attracted to a simple model for these processes, namely the influenza virus and the red blood cell or erythrocyte. The influenza virus has the capacity to agglutinate erythrocytes that can serve as a useful method to titrate the amount of virus in a fluid. This obviously suggests that each virion has many attachment molecules, thus being capable of binding several erythrocytes together. Today we know this attachment molecule as the haemagglutinin. Burnet observed that if virions and red cells were incubated together, the agglutination was gradually reversed. Moreover, these erythrocytes could now no longer be agglutinated by fresh virus. The results suggested the possibility that an enzyme was destroying the erythrocytes' receptors, and so the term receptor-destroying enzyme (RDE) was coined. Ada set about studying the virion-erythrocyte interaction in his usual systematic way.

First, the electrophoretic mobility of human erythrocytes was decreased following adsorption and elution of influenza A (PR8) virus from 1.32 to 0.5–0.6 μm/second/volt/cm.[1] The decrease was dependent on virion concentration, suggesting that it was the result of enzymic action of the virus. Burnet had previously found that extracts of *Vibrio cholerae* bacteria contained RDE, and red cells treated with this preparation had even lower mobility, viz 0.17. Another feature of this work was the observation that different strains of influenza and different other viruses such as mumps and Newcastle disease virus acted on receptors to varying degrees, creating a gradient. Ada found that the reduction in electrophoretic mobility caused by various viruses mirrored exactly the loss of agglutinability. The reasons for this gradient were not immediately apparent. Next, French and Ada[2] showed that erythrocytes could repair their surface to a certain extent.

Ada was uncomfortable working with crude extracts of RDE so set about a series of studies designed to purify the bacterial enzyme. The first attempts[3] used adsorption to and elution from erythrocytes followed by ammonium sulphate fractionation, resulting in 500-fold purification of the crude extract. Final success was to come much later, with the addition of hydroxylapatite column fractionation and crystallisation.[4] This pure RDE was in fact neuraminidase. Of course, it was from a bacterial source. Ada's early research assistant in this work was Graeme Laver, who later left to undertake a biochemistry course at the University of Melbourne. In due course, Laver moved to the Australian National University. There he purified influenza virus neuraminidase and produced the first crystals, but they were of insufficient quality to permit X-ray crystallographic analysis. In Melbourne, Varghese and Colman used a different virus isolate that provided better crystals, and they solved the neuraminidase structure. This permitted Mark von Itzstein to design and synthesize an inhibitor that became the successful anti-influenza compound, Relenza. Ada's other studies of this period concerned themselves with the stimulation of neuraminidase production in bacteria and with a comparison of neuraminidases from various tissues and species.

Structure of Influenza Virus Proteins and Nucleic Acids

In the early 1950s little was known of either the proteins or the nucleic acids of viruses. Ada's work on virus proteins was frustrating. The methods for purifying viral antigens, separating them from contaminating host proteins, and analysing them were simply not robust enough. Ada used anti-influenza antibodies to interrogate complement-fixing antigens of the virus isolated from infected chick embryo lungs.[5] Immune

precipitates were treated with various buffers and solvents, of which chloroform proved the most satisfactory, and then extracted with methanol. The solution was analysed by electrophoresis and ultracentrifugation. While a single major peak was identified by both methods, immunodiffusion studies showed clear heterogeneity. Ada concluded that further attempts at purification by physical methods were unjustified.

A collaboration with Alfred Gottschalk proved much more successful.[6] Gottschalk is generally credited with discovering the influenza virus glycoproteins through years of painstaking work on influenza virus neuraminidase. Ada's contribution was to obtain accurate information about the sugar components of the influenza virus particle. The oligosaccharide portion of the viral glycoproteins consisted mainly of galactose, mannose, fucose and glucosamine. Small amounts of galactosamine were also present. Later work showed N-acetyl neuraminic acid to be an essential component of the neuraminidase glycoprotein.

By far the most important contribution Ada made to our understanding of influenza virus was to show that it was an RNA, not a DNA virus. In the early 1950s there was no consensus about the nature of viruses. Were they in fact independent microorganisms? After all, they could only survive inside other cells. Burnet believed firmly that viruses were the simplest forms of life, but the nature of their genetic machinery was unknown. Right up to the time of Watson and Crick (1953), some scientists believed that genes were protein in nature. It was against this background that Ada began his study of the nucleic acid content of influenza virus in 1953. Prior research by others had been confusing, some investigators finding only DNA in influenza samples, others substantial amounts of both DNA and RNA and still others a majority of RNA but some DNA. Recall that at that time RNA was still referred to as yeast nucleic acid and DNA as thymus nucleic acid.

Ada set about purifying influenza virus preparations to at least 90% purity.[7] After extensive defatting, virus samples were extracted with 10% NaCl in a boiling water bath. The residue was then subjected to perchloric acid extraction. A sugar reaction showed that there was only ribose and negligible quantities of deoxypentose present, thus RNA but no DNA.

Is this RNA the virus's genetic material? Methods exist for producing influenza virus preparations that possess high haemagglutinating activity but low infectivity. Ada found that such 'incomplete' virus preparations had much less RNA, consistent with the view that RNA was the material that allowed the virus to replicate.[8] Unfortunately, attempts to prepare a pure RNA preparation that was infectious proved unsuccessful. However, in collaborative experiments with S. G. Anderson, infective RNA was prepared from Murray Valley Encephalitis-virus,[9] cementing the principle that RNA could act as a set of viral genes. One fruitful outcome of this work was the development of a purification method suitable for most viruses.[10] It involved treatment of infected tissue extracts with protamine, ultracentrifugation, adsorption to and elution from hydroxyl apatite and a second ultracentrifugation. Recovery of virus infectivity was ~50%.

Ada's demonstration that influenza was an RNA virus was multiply confirmed and universally accepted. We now know that the genome is segmented into separate pieces of RNA, permitting the possibility of recombination (reassortment) when two different viruses infect a single cell.

Studies Tracing the Spread of Antigen Through the Lymphoid System Following Injection

The year 1957 was a watershed in the history of the Walter and Eliza Hall Institute. Burnet, one of the most distinguished virologists of his era, and a multiple nominee for the Nobel Prize, decided to shift the interest of the Institute from virology to immunology. Part of the reason was that virology was becoming more and more molecular, and reliant on tissue culture. Burnet's beloved chick embryos for growing viruses were being sidelined as scientific tools. Though Burnet had a good, small, biochemical unit under Ada, he was himself uncomfortable with the many instruments and technologies required for biochemical work. On the more positive side, Burnet's theoretical work in the field of immunology had been well received, especially his postulates about how the body discriminated between 'self' and 'not self', forming antibodies to foreign materials but not to components of the body itself

(at least in health). Some of his predictions about this phenomenon of immunological tolerance to 'self' had been validated experimentally by Peter Medawar's group[11] and resulted in Burnet and Medawar sharing the Nobel Prize in Physiology or Medicine in 1960.

In this same year, 1957, Burnet came up with a modification of Niels Jerne's 'natural selection' theory of antibody formation.[12] The dominant paradigm of the time was the direct template hypothesis, which held that antibody was molded on an antigen template much as soft plastic on a dye. However, this offended emerging concepts of protein formation that held that the sequence of amino acids in a protein was determined by the gene sequence, and further that the sequence determined patterns of folding. Jerne had speculated that the different shapes of antibodies with millions of different variants were generated spontaneously without a requirement for antigen. He saw antigen as somehow catalysing formation of more of the particular antibody with which the antigen had united. Burnet, taking a lead from David Talmage, saw the different antibodies as receptors on the surface of lymphocytes, such that one cell had only one kind of antibody receptor and thus could, after stimulation with antigen, form only one antibody. He termed this the clonal selection theory.[13] Gathering evidence for or against this theory became a major preoccupation of the Institute. One of the authors of this article (GN) was able to use micromanipulation techniques to show that one cell from a multiply immunised animal could indeed form only one antibody,[14] a first hint in favour of clonal selection.

Burnet did not force any of his senior people to leave virology and Ada continued in this field until 1962. However, it became an increasingly lonely exercise. Henry Holden and Alfred Gottschalk had retired, Stephen Fazekas had moved to Canberra to work in Fenner's department, Gray Anderson moved to London and Eric French took a senior position with CSIRO. In 1962, Ada decided to change direction and to see if he could make a contribution to immunology. Methodical as always, he retreated to the library to find out how workers were tackling the basis of antibody formation. He became interested in the fate of injected antigen (chiefly in mice). Most investigators had used large amounts of antigen in order to find any after it had been distributed through the body. Unsurprisingly, much antigen ended up in scavenger cells (macrophages), for example, in the liver. It was difficult to see how this could have contributed to the induction of antibody formation. If an antigen such as ferritin, identifiable by electron microscopy, was injected, some particles could be seen within plasma cells, the chief antibody formers, giving comfort to proponents of the direct template hypothesis. But Ada had been used to working with very small quantities of virus and thought it would be interesting to determine where critically small amounts of antigen would end up. If a powerful antigen capable of causing antibody formation when injected in nanogram amounts could be suitably traced, its fate might reveal some of the secrets of immune induction.

Ada was kind enough to discuss his ideas with one of us (GN). Thus began a productive five-year collaboration (Fig. 2). The flagellae of *Salmonella* bacteria constitute a very strong antigen. The key component protein, flagellin, can be readily purified and radioactively labelled, in the first experiments with the radioisotope iodine^{131} (I^{131}). Moreover, flagellin can be prepared either as a monomeric protein of molecular weight of ~40,000 daltons or as a polymer of an average of 300 monomer units resembling the original flagella in shape. In our first experiments we used I^{131} but soon I^{125} became available that released less energetic particles when it decayed, giving more precise localisation in autoradiographs of tissues. Ada was able to devise a very gentle method of iodination, and to use carrier-free isotope, giving the labelled protein very high specific activity. Largely at the suggestion of Walter Spector, we chose rats rather than mice as the experimental animal as the lymph nodes would be larger and more easily dissected out and histologically sectioned. Following footpad inoculation, 10 ng of flagellin was immunogenic and even 1 ng caused antibody formation in some rats. One day after injection, only about one per cent of antigen could be found in the lymphoid organs, and calculations showed that less than 10^9 molecules of flagellin could induce antibody formation. The lymph node draining the injection site, the popliteal node, retained only 0.04% of the antigen at 48 h, and the specific activity per mg of tissue was 10 times less for the next lymph node up the chain, the para-aortic node. With intact flagella, as few as 10^5 retained particles

Figure 2. Peter Doherty, Bob Blanden, Rolf Zinkernagel and Gordon Ada attending the 11th Frank and Bobbie Fenner Conference in Canberra in 2003, which celebrated the retirement of Bob Blanden (photo provided by the authors).

would prove to be an active immunogenic dose.[15]

The cellular localisation of flagella in the popliteal lymph nodes showed striking results.[16] As expected, macrophages in the medullary sinuses of the lymph node were labelled but, surprisingly, so were rounded areas lying superficially in the lymph node cortex known as lymphoid follicles. Closer examination showed the antigen to be associated with discrete cells, possessing long dendritic arms extending between the lymphoid cells. Within about three days, rapidly dividing, large lymphoid blast cells had collected adjacent to the antigen depot, and within five days a small, typical 'germinal centre' had appeared that continued to grow for at least four weeks, significant amounts of antigen being retained for at least twelve weeks. Within the medulla of the node, great numbers of plasmablasts and plasma cells appeared. These did not exhibit labelling. A wide range of proteins were studied with respect to follicular localisation. All antigenic substances labelled follicles, though to varying degrees. Minimally antigenic proteins, such as calf gelatin, were minimally positive and rat serum albumin, rat haemoglobin and rat erythrocytes (self antigens) were entirely negative. It thus appeared that follicular antigen-capturing cells (later termed follicular dendritic cells or FDC) somehow possessed the capacity to 'recognise foreignness'. This result anticipated by thirty years the discovery of the Toll-like and other receptors capable of recognizing pathogen-associated molecular patterns. The only 'self' protein found to localize in follicles was rat **γ** globulin, due to FDC possessing Fc receptors.[17] One confusing finding was that when rats were rendered tolerant by repeated injections of small doses of the flagellar antigen this did *not* result in labelled antigen behaving like a 'self' molecule but rather showed marked follicular localization.[18] We can now presume that this was due partly to Toll-like receptor 5 that can recognize bacterial flagella, and partly to the antigen complexing with antibody, as the tolerance was not complete, thus favouring Fc-mediated attachment.

Further insights into FDC function were obtained through electron microscopic autoradiography.[19] This demonstrated that, whereas macrophage-associated antigen was inside intracellular vacuoles (phagolysosomes),

FDC-associated antigen was essentially extracellular. Antigen was retained on the surface of the thin, dendritic processes interdigitating between lymphoid cells. FDC had the capacity to hold antigen extracellularly for many months, permitting interaction with receptors on surrounding lymphocytes. As the germinal centre developed, an electron-dense material accumulated around the dendritic processes. This represented antigen–antibody complexes. Now the only antibody receptors on germinal centre B-cells that could gain access to follicularly located antigen were those with a higher affinity than the mean of serum antibody. Stimulation of such B-cells with mutated antibody genes conferring higher affinity could, over time, lead to progressive affinity maturation of the antibody response. Later work rendered this a highly likely explanation.

Through micromanipulation it was possible to identify anti-flagellar antibody-forming cells and then to place each cell on a marked area on a gelatin-coated microscope slide. Following drying and fixation, slides were dipped in photographic emulsion, dried, exposed in the dark for 60 days (just over the 58 day half-life of I^{125}), developed, fixed, stained and examined at 1250-fold magnification. The number of developed silver grains overlying each cell and an equal area of background in the same microscopic field were recorded. As flagellin had been heavily labelled, a mean antigen content of four molecules of flagellin per cell would have yielded a net mean grain count around one. In fact, the results from 216 single antibody-forming cells from 15 separate experiments yielded a mean net grain count of –0.4, not significantly different from zero. Similarly, examination of sections comprising many thousands of plasma cells failed to find any that were unequivocally labelled. Given that there are very many polyribosomes in each antibody-forming cell, these results made it highly improbable that antigen was acting as a direct template within these cells. Thus, these studies provided unequivocal evidence that the template theory for antibody production was untenable.[20]

Evidence in Favour of the Clonal Selection Theory Through 'Hot Antigen Suicide'

Ada was much impressed by the work of David Naor and Dov Sulitzeanu[21] who showed that when a preparation of lymphocytes was exposed to a radiolabelled antigen at 4°C, only a very small proportion of cells became heavily labelled, just what would have been expected from Burnet's clonal selection hypothesis where some cells were, by chance, equipped with antibody receptors capable of recognizing that antigen. A functional test was required. Would I^{125}-labelled antigen binding specifically to a lymphocyte cause enough radiation damage to that cell to prevent later effective stimulation towards antibody production? To test this, cell preparations were held at 4°C for a day or two with labelled antigen and were then injected into mice of the same inbred strain whose own immune system had been crippled by whole body irradiation. Then these host mice were challenged with the relevant antigen (unlabelled) and a second (irrelevant) antigen. The results were clear cut. The recipient mice made much less antibody to the specific antigen than to the control. Almost certainly, the unimmunized lymphocytes capable of binding a given antigen were the precursors of the cells that, following immunisation, made the corresponding antibody.[22]

In 1969 and 1970, the above substantial body of work was consolidated into a book, published in 1971.[23]

MHC Restriction and the Doherty-Zinkernagel Nobel Prize

In 1968, Ada received an invitation from Frank Fenner offering him the Chair of Microbiology at the John Curtin School of Medical Research at the Australian National University. This had been Fenner's own department before he was promoted to become Director of the School. Ada accepted, and moved to Canberra in late 1968 (see photograph on first page taken a few years later in 1971).

Initially when Ada joined the Department of Microbiology he was met with some hostility from the academic staff. At that time the Department was dominated by virologists and they feared that Ada, who was an enthusiastic promoter of immunology, would oversee a take over of the Department by immunologists, as had recently occurred at the Hall Institute. They were mistaken. In fact, Ada's plan was the exact opposite as he aimed to encourage virologists and immunologists to work together and

study the immune response against viral infections. He did recruit several excellent immunologists soon after taking over the Department but virology continued to be a strength of the Department and a perfect environment was created that encouraged collaboration between the two disciplines.

This collaborative environment was enhanced further by the establishment of the weekly 'Bible Class'. Ada was not a religious man and the name had no religious connotations. It was just an amusing name for an informal group that discussed science. Nevertheless, the name caused confusion with some new academic staff thinking it was compulsory religious instruction being imposed upon them! The Bible Classes occurred at lunchtime every Monday when academic staff and students crowded into Ada's office. Everyone was invited, including researchers from other Departments of the JCSMR, the only entry requirement being to bring a chair. Those who attended the meetings, from the most junior student to the most senior Professor, were expected at some stage to make a presentation, with Ada often recruiting contributors with little notice. The presentations were extremely informal and ideas were communicated with minimal visual aids—a whiteboard or scribbling on butcher's paper was all that was available. The subject of the presentations was wide ranging, latest experimental data being the most popular subject, but discussion of 'left-field' ideas or challenging recent publications was also quite common.

It was at a Bible Class in September, 1973, that Peter Doherty and Rolf Zinkernagel first presented their paradigm-shifting research on the role of the major histocompatibility complex (MHC) in the recognition of virus infected cells by cytotoxic T lymphocytes (CTL). Their experiments showed that lymphocytic choriomeningitis virus (LCMV) specific CTL could only recognize and kill LCMV-infected targets if they shared some MHC antigens with the target cells. They suggested that the CTL recognized on the infected target cell surface a virus-induced change in MHC molecules, possibly resulting from the virus forming a complex with the MHC molecules, a change that they subsequently termed 'altered self'. Up until this time the MHC had been defined as a highly polymorphic region of the genome that was responsible for the vigorous rejection of grafts between individuals of the same species when they expressed different MHC alleles, but the biological relevance of this phenomenon remained a mystery. The Doherty and Zinkernagel discovery provided an explanation for MHC-mediated graft rejection and explained why the MHC was so polymorphic, this polymorphism ensuring that there would always be members of a population that are highly effective at producing CTL against almost any intracellular pathogen.

One of the authors of this memoir (CRP) had the good fortune to be present at this historic Bible Class. Although the experimental evidence to support the MHC restriction hypothesis was not complete at that time it was obvious to everyone in the room that a major scientific discovery had been made. Additional experiments by Doherty and Zinkernagel in subsequent months reinforced their initial findings and resulted in a highly influential paper being published in *Nature* in April 1974[24] that eventually resulted in them being awarded the Nobel Prize in Physiology or Medicine in 1996 (Fig. 3).

Based on the above it is clear that, in addition to the considerable number of scientific discoveries made by Ada, the research environment he created in the Department of Microbiology, JCSMR, nurtured outstanding advances in immunology and is one of his finest scientific achievements. The research in viral immunology by Doherty, Zinkernagel, Blanden and others in the Department of Microbiology also prompted Ada to return to virus research, particularly of influenza. However, in this case, rather than studying influenza virus structure he investigated the immune responses against viruses. For example, he showed that virus-specific CTL could recognize and kill virus-infected target cells 1–2 h after infection, which was many hours before infectious progeny virus was released from infected cells.[25] He also performed a long series of experiments probing the role of $CD4^+$ helper T-cells in anti-influenza virus immunity.

Work with the World Health Organization

WHO had major interests in immunology and in 1969 the Head of its immunology unit, Howard Goodman, asked Gordon Ada to join a meeting of eminent immunologists to review recent

Figure 3. Frank Fenner and Gordon Ada enjoying a 'retirement' cocktail in a courtyard of the old John Curtin School of Medical Research in 2005 (photo provided by the authors).

advances. He was the rapporteur for that meeting, starting an association that was to continue for the rest of his professional life. At about the same time, Ada also began work with a WHO-affiliated organization, The International Agency for Research on Cancer or IARC in Lyon, France. First, he helped on IARC's Fellowship Selection Committee for three years and during the third year he joined IARC's Scientific Board. Through this experience he gained insights into the great importance of epidemiology in cancer research that were to stand him in good stead in later years when he became profoundly interested in HIV/AIDS epidemiology. Ada ended his six-year stint with IARC as Chairman of the Scientific Board.

Within WHO itself, based at the Geneva headquarters, medical research received only modest support from the regular budget. Accordingly, research enthusiasts within the Organization set up programmes that concentrated on research and sought their budgets separately. The first of these special programmes was HRP, the Human Reproduction Programme under Alex Kessler and the second was TDR, the UNDP/World Bank/WHO Special Programme on Research and Training in Tropical Diseases, under Adetokunbo Lucas. This very successful programme, launched in 1976, is still active today. Initially it concentrated on five parasitic diseases (malaria, schistosomiasis, filariasis, trypanosomiasis, leishmaniasis) and one bacterial disease (leprosy). Ada was asked to join the Scientific and Technical Advisory Committee, and served two three-year terms on it. Ada was able to neutralize an initially critical attitude toward recombinant DNA research (genetic engineering) and also contributed greatly to technical reviews of malaria, leprosy, schistosomiasis and field research.

In 1981, Ada was appointed to represent Australia on the most senior and prestigious WHO research committee, the Global Advisory Committee on Medical Research (GACMR), later changed to Health Research. Here again he acted as a forceful advocate for recombinant DNA research and also for the important role that monoclonal antibodies could play. He was involved in efforts to transfer these and other advanced technologies to researchers

within developing countries. While he was on GACMR itself for the standard four-year term, this extra work extended his association with GACMR for eight years. All this exposed Ada to world-renowned figures such as WHO Director, Halfdan Mahler, the Swedish Nobel Laureate, Sune Bergström and cancer pioneer Dennis Burkitt.

The next important WHO task was chairing a new body, the overseeing committee of an initially small Programme for Vaccine Development (PVD). The first leader of this was Fakhry Asaad, and the next Paul-Henri Lambert. Ada's committee was felicitously termed SAGE, the Strategic Advisory Group of Experts. PVD and SAGE grew progressively and acted as a focal point for all vaccine development within WHO. It studied adjuvants, recombinant live vaccine vectors, biodegradable controlled-release microcapsules for vaccine proteins, mucosal immunity and T-cell epitopes. It also catalysed interactions with industry on specific new vaccines. SAGE functions essentially as the research arm of all WHO immunization programmes, including the Expanded Programme on Immunization (EPI) and the Global Poliomyelitis Eradication Campaign. When Ada finished his chairmanship of SAGE in 1990, a distinguished virologist, Fritz Deinhardt, took over but unfortunately he died within a year and one of us (GN) became Chairman of SAGE for an extended period. Ada also had significant interactions with the Human Reproduction Program (HRP) and with the Global Programme on AIDS. He served on the Western Pacific Regional Advisory Committee for Health Research for a further four years.

This monumental series of contributions to WHO helped as a fitting introduction to Ada's heavy involvement in HIV/AIDS following his retirement from the John Curtin School.

Contributions to the Australian Academy of Science

Gordon Ada was elected to the Fellowship in 1964 at a time when only six scientists per year were so honoured. This was largely because of his influenza work, particularly the proof that influenza was an RNA virus. He had two stints on a Sectional Committee, 1969–72 and 1981–4. He served as a Council Member 1972–5 and as Vice-President B 1974–5. During this period came an exciting three-week visit to China. There had been reciprocal visits between the Academy (AAS) and its Chinese counterpart, the Academy Sinica (AS) in the early 1960s, but when the AS reinvited the AAS in the late 1960s, Burnet as President declined because the regrettable Cultural Revolution was in full swing. In 1973, the Chinese tried again, this time via a joint invitation to the AAS and the Australian National University. Sir Rutherford Robertson, then President of the AAS, accepted and Ada was chosen as one of eight members of the delegation. While admiring much of the scientific progress in China, Ada expressed distress at the degree to which the AS was forced to conform to official government views. Representations from the AAS and the Royal Society to grant the AS greater independence failed utterly.

Clearly successful as an Australian representative, Ada was asked to become Foreign Secretary of the AAS in 1977. Back he went to China, initiating a scientific exchange programme between the two Academies. The Cultural Revolution had just ended, the atmospherics were much better, and a highly successful agreement was negotiated. Further highlights of Ada's tenure as Foreign Secretary were a strengthening of the already solid bonds with the Royal Society of London and collaboration with the Japanese Society for the Promotion of Science. Ada also attended meetings of the International Council of Scientific Unions (ICSU) and was appointed to the ICSU Council, but this involvement was handicapped by a paucity of Australian Government funding.

Ada's time on the AAS Council coincided with a turbulent period in the history of molecular biology. Distinguished US molecular biologists, most prominently later Nobel Laureate Paul Berg, drew attention to the potential hazards of some recombinant DNA research, for example recombinant *E. coli* bacteria with toxin genes in them, or with carcinogenic potential. Ada was asked to chair an AAS committee to study such conjectural hazards and to report on appropriate actions. One important result was that two Fellows, Jim Peacock and Jim Pittard, were sent to the famous Asilomar Conference in 1975 that developed a set of guidelines under which recombinant DNA research could proceed. They reported back and an AAS Standing

Committee was formed in Australia, which successfully supervised this area of research for many years until eventually a formal Government advisory regime was initiated, led with distinction by now Academy Chief Executive Sue Meek. Because of this careful and skilful work, Australia was able to conduct much important research without the dissention and bitter strife that accompanied genetic engineering in the USA and many other countries. Admittedly, this situation was more true for animal rather than plant experiments. Ada's many contributions to the Academy over this long period have won him great respect and gratitude from the Fellowship.

Retirement

In 1986, Ada relinquished his Headship of the Department of Microbiology, JCSMR, to Bob Blanden and retired when he turned 65 years old in December 1987. But retirement from the ANU did not result in the end of his scientific career. Shortly before retirement, Ada was invited to take up a six-month consultancy in vaccine development with WHO in Geneva, an offer that he accepted and took up in the first half of 1988. He was also approached by Dr Noel Rose who offered Ada a retirement position at the Johns Hopkins School of Hygiene and Public Health, Baltimore, commencing in July 1988 that Ada also accepted and occupied until 1991. Of particular significance at that time was a plenary lecture entitled 'The prospects for HIV vaccines' that Ada delivered at the Fourth International AIDS Congress in Stockholm in May 1988. The collective wisdom of immunologists supported the development of vaccines that induced neutralizing antibodies against HIV. Ada, however, stunned the 8,000 strong audience by presenting seven reasons why such a vaccine would not work, in particular because HIV can extremely rapidly generate escape variants from any neutralizing antibodies that can be induced. Instead he advocated the development of vaccines that induced HIV-specific CTL against internal HIV antigens that would be likely to be more highly conserved. Although his proposal was initially viewed with some scepticism, particularly by vaccine manufacturers, in the ensuing years his proposal became widely accepted.

Following his arrival in Baltimore in mid-1988, soon after the Stockholm HIV conference, Ada was made an Associate Director of a recently established Center for AIDS Research, eventually becoming the Director of the Center. He was also invited by the National Institute of Allergy and Infectious Disease, Washington, to be a member of the Division of AIDS (D.AIDS), an organization to which he continued to contribute very actively until 1995. Despite an effective HIV vaccine still not being available at the time of writing this memoir there is no doubt that Ada has made a considerable contribution to our understanding of the basic requirements for such a vaccine.

In 1991, Ada returned to Australia with his wife Jean and became a Visiting Fellow in the Viral Immunology Group, Division of Cell Biology, JCSMR. He was also immediately appointed Chairman of the Australian HIV Vaccine Working Group. Soon after his return to Australia Ada was made, in 1993, an Officer of the order of Australia (AO) for service to medicine in the field of immunology and international health.

In his retirement, Ada remained a passionate advocate for vaccination, in 2001 co-authoring a book for parents, educators and students entitled *Vaccination: The Facts, the Fears, the Future*[26] and for many years talking with community and school groups about the virtues of vaccination. He found time in his retirement to return to his passion for sailing and could be often found sailing on Lake Burley Griffin in Canberra. He also came to work in his office at the John Curtin School almost every day until 2007 when ill health made it difficult for him to continue. He, often with Frank Fenner, attended most School and Departmental seminars and was always available at morning tea and lunchtime to discuss science with all staff and students. In fact, during this period Gordon acted as a great mentor for countless students and postdocs (Fig. 4).

Overall Gordon Ada will be remembered as a superb experimentalist, as a great mentor of students, as a remarkable facilitator of scientific interactions, as a passionate advocate of science to the community and last, but certainly not least, as a good colleague and friend.

Honours and Awards

Fellow of the Australian Academy of Science (FAA), 1964

Officer of the Order of Australia (AO), for service to medicine in the field of immunology and international health, 1993

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