Translating Molecular Insights in Autoimmunity into Effective Therapy

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Abstract

Autoimmunity and the pathogenesis of autoimmune diseases were a major focus of the Walter and Eliza Hall Institute, where I started my research career. After my initial studies on immune cell culture and immune regulation, I returned to an analysis of the pathogenesis of human autoimmunity in London. Linking upregulated antigen presentation to autoimmunity led to an investigation of the role of cytokines in rheumatoid arthritis (RA), in collaboration with Ravinder Maini. These experiments defined the concept of a TNF-dependent cytokine cascade driving the manifestations of RA, which led to successful clinical trials of anti-TNF monoclonal antibody in RA patients, heralding a major change in medical practice. This success was made possible by enthusiastic support from many laboratory and clinical colleagues and taught us that cytokines are important rate-limiting steps and hence good therapeutic targets. My current scientific challenge is exploring the hypothesis of whether all major medical needs can be approached via cytokine blockade.

GROWING UP IN AUSTRALIA

Postwar France was poor, and despite my Polish father's French accountancy degree, life was difficult. My Jewish parents thus sought greater opportunities elsewhere. My father had two cousins, both doctors, one in New Haven, Connecticut, in the United States, and the other in Melbourne, Australia, and they both filed immigration papers for my family. The Australian papers came a month before the U.S. papers. Who knows what might have happened had U.S. bureaucracy been speedier.

As an eight-year-old, a month's journey by ship to Australia was an adventure going into the unknown, to a land of kangaroos and much promise, visiting the pyramids en route. Learning English in a land welcoming immigrants was not a major hurdle. Immigrants have a very strong motivation to work hard and succeed. As a child, the image of my father coming home tired from his work as an accountant in a factory, to study anew for his accountancy degree, as his French qualification was not recognized, had a profound impact on me. Once qualified, he built up a prospering sole-proprietorship serving other immigrants. Having helped balance books of accounts on weekends and holidays, I found the thought of following in his footsteps far too boring! My father's cousin, more flamboyant, was a doctor, and that seemed to my older brother and me a more challenging and possibly more satisfying profession, so we both became medical students at the University of Melbourne.

Medical studies opened up new vistas. Some courses were painstaking and meticulous—five terms of learning and regurgitating anatomy is a chore that is mercifully no longer imposed—but others were exciting and challenging. Biochemistry teachers encouraged successful students to read more widely. My first exposure to the *Annual Reviews of Biochemistry* was an eye-opener, illustrating the questioning and uncertainties of emerging ideas and knowledge, rather than the definite facts and platitudes that we students usually received in lectures.

Microbiology provided my first glimpse of immunology, with eight lectures on serology taught by a microbiologist on the use of antisera to diagnose infections. A few years later, I learned that this course bore no resemblance to the major discoveries about cellular immunity being made contemporaneously on the other side of the Sydney Road from the university campus, at the Walter and Eliza Hall Institute of Medical Research, by luminaries Jacques Miller, Gus Nossal, and others, who eventually became my mentors.

Clinical studies were challenging for a young, immature student like me, who at 17 started medicine straight from high school. We had already experienced death early in our studies, by working with cadavers in anatomy. The University of Melbourne clinical studies were performed in two hospitals: at the Royal Melbourne Hospital, over the road from the university, which took a large group of students, and at St. Vincent's Hospital in Fitzroy, which took one-fifth the number of students that Royal Melbourne did.

I suspect that I am impatient. I got married while still a medical student, and my son was born while I was working in hospital and my daughter while I was completing my PhD. I made what in retrospect was a pivotal decision: to go to St. Vincent's, the hospital with fewer students, because I was impatient to learn clinical medicine more rapidly by seeing the most interesting patients, which I hoped being part of the smaller group would allow. It was indeed easier to learn clinical medicine, but I paid the price later when, after qualification, I and the other young doctors had to see all comers and not just the interesting cases. St. Vincent's was in a poor, hard-drinking suburb of Melbourne, so almost 50% of patients arriving in casualty (emergency room) were chronic alcoholics, who have neither the most pleasant demeanors nor the most intellectually challenging problems. I therefore began exploring research opportunities earlier than I might have if I had chosen the more academic hospital.

SCIENCE IS DIFFERENT FROM MEDICINE: GOLDEN YEARS AT THE WALTER AND ELIZA HALL INSTITUTE

The Walter and Eliza Hall Institute of Medical Research (WEHI), across the road from the university, was made famous by the Nobel Prize winner Sir Frank MacFarlane Burnet, who had been a pioneer both in virology and subsequently in immunology. The institute had recently appointed a young and dynamic new director, Gustav Nossal. I went to meet him and to see the institute. The contrast with the rest of the university was stark: WEHI was clearly in a different league. Gus accepted me as a PhD student for the following year, to work on new techniques of tissue culture for generating immune responses in vitro. Little did I know that I had applied far too late, but Gus's intuition was to make an exception and seek another PhD studentship for me.

When I arrived in February, after a summer break recovering from the stresses of endless on-call rotations in the hospital, an arduous apprenticeship compared with today's European Union work regulation–restricted hours, I was greeted by Erwin Diener, the Swiss scientist Gus had chosen to supervise my first tentative steps into science (see **Figure 1**). The project was wonderful, optimizing in vitro lymphoid cell cultures that were independently being developed in the United States by Bob Mishell and Dick Dutton (1) and at WEHI by John Marbrook (2) and Erwin Diener (3).

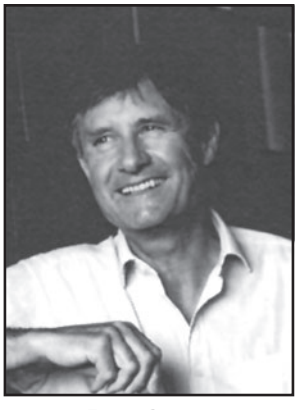
The project was wonderful because of its potential influence on virtually all aspects of immunology. In vitro experiments are truly reductionist, and if that is to your taste, all the elements involved can be controlled: cells purified and quantitated, antigen concentration maintained precisely, other stimuli controlled. But this control comes at the same price as all the reductionist science still popular today (e.g., gene knockouts): Concepts generated in one precise circumstance often do not extrapolate to complex and nonreductionist reality.

With Erwin, I started to improve the current culture methods. It was already possible to generate antibody production from mouse spleen cells. The antigen used, *Salmonella* flagellin, was popular at WEHI, having been used by Gus Nossal and Gordon Ada to help validate Burnet's clonal selection theory (reviewed in 4). This antigen had been used to demonstrate that one cell produced only one antibody,

Mentors

Gustav Nossal

Jacques Miller



Erwin Diener

Figure 1

My mentors at the Walter and Eliza Hall Institute of Medical Research, Gustav Nossal, Jacques Miller, and Erwin Diener.

even from lymph nodes of multiply immunized mice. Assaying flagellin immunity was laborious: Cell suspensions were incubated with bacteria, which adhered to the antibody-forming cells. The suspension was plated on agar, and colonies were grown for a few hours to enable discrimination of cells that had bound multiple bacteria from single bacteria (5). The simpler single-cell assay was the hemolytic plaque assay, developed by Niels Jerne (6) and his collaborators. It used complement-mediated lysis, either on agar or between glass slides. An improvement I engineered was to convert the cumbersome bacterial assay to a plaque assay, coating the red cells with protein. For multipurpose use, I developed the technique of using anti-sheep red cell Fab fragments (7), which could be derivatized with haptens, e.g., DNP or proteins, such as, for example, myelin basic protein (MBP), work I did in collaboration with fellow PhD student Vanda Lennon (8).

Initially, we performed these cultures on a small scale, 10–20 flasks permitting 3–6 groups, producing 3–6 sets of data to compare. But as techniques improved, many more questions arose, and so the glassware proliferated, as did the need for more washing up, more media, more sera incubators, etc. I needed more resources. Thus, I learned early at WEHI the virtue of collaborations, pooling intellectual and material resources to enhance scientific productivity. Effective collaboration has been a key part of WEHI's success as an international scientific powerhouse over its long history. Erwin had two laboratory technicians, and as he was a reflective scientist, not prone to an excessive number of experiments, his technicians were encouraged to assist me. As a beginning PhD student, I found this to be a wonderful situation, as was having Erwin's patient help in developing my scientific writing skills.

Jacques Miller was at his magnificent prime when I started at WEHI. With Graeme Mitchell, he had just published a series of three landmark papers (9–11) documenting that thymus-derived lymphocytes did not in themselves make antibody or develop into antibody-forming cells, but rather interacted with and activated bone marrow–derived antibody-forming cell precursors. He had also recently been elected a fellow of the Royal Society, ahead of the other two scientific giants at WEHI, Gus Nossal and Don Metcalf. Jacques Miller's unit studied the function of thymus-derived cells, later renamed T cells by Ivan Roitt et al. (12, 13), whereas Gus Nossal's unit, where I worked, studied antibody formation from B cells, bursa-equivalent or bone marrow–derived lymphocytes.

Growing up in Australia inevitably engendered the love of playing sport. There were no tennis courts in the vicinity of WEHI, but there were squash courts buried in the bowels of the Royal Melbourne Hospital. I played regularly with Tony Basten, a postdoc in Jacques's unit, and so over sweat and drinks we evolved a collaboration to try to recreate in tissue culture the T-B interactions that Miller and Mitchell had reported in irradiated mice.

Tony provided a series of irradiated mice repopulated with thymus cells only, a source of relatively pure T cells (9), and I put them in culture with a variety of other populations, usually adult thymectomized bone marrow–grafted mice (14) that Tony had also provided, where B (but not T) lymphocyte repopulation takes place. To study the process in more detail, I developed a variant of the Marbrook-Diener culture system that I had been using to study a variety of immunological processes in vitro, including immunological tolerance. This is illustrated in **Figure 2** (15). It permitted separating the two cell populations to assess whether direct cell contact or cell-free mediators were sufficient. The results we obtained (16) were published back-to-back with Anneliese Schimpl/Eberhard Wecker's (17) results generated in the alternative Mishell-Dutton culture system.

Other scientific interests that I have pursued subsequently were nurtured at WEHI. Ken Shortman was the head of the Biochemistry Unit, but his main focus was cell separation: how to purify cell subsets. Using these techniques enabled me to study lymphocyte-macrophage interactions, the

process now known as antigen presentation, an acknowledged vital key step in the generation of immune responses. Macrophages are very adherent, so they can be enriched by adherence, and lymphocytes, relatively nonadherent, can also be enriched through this technique. But a more physiological approach was to use recirculating cell populations in vivo enriched in lymphocytes, and access to these cell populations is possible by thoracic duct drainage. John Sprent, a fellow PhD student in Jacques's unit, was working extremely long hours collecting such cells after the tricky surgery to cannulate the duct and prevent the tiny tube from blocking up. He generously provided these cells, and we showed that thoracic duct cells alone were not able to respond to a particulate antigen, sheep red cells in vitro, unless supplemented by adherent macrophages (18).

Vanda Lennon was also my contemporary at WEHI, a PhD student in the Clinical Research Unit headed by Ian Mackay. She studied experimental allergic encephalomyelitis, assisted by Patrick Carnegie. In these animals, we generated autoantibodies to MBP and devised a project to use MBP-coated red sheep cells to detect where the antibody-forming cells to MBP were present in animals. We duly found them in the brain (19).

In hindsight, these research interests nurtured at WEHI were recombined in 1983 to help me conceive of a new hypothesis linking upregulated antigen presentation and autoimmunity, triggered by the immunohistological data of Franco Bottazzo (20) and others [e.g., Klareskog & Wigzell (21)] that there was augmented HLA class II expression in autoimmune disease tissues, such as the thyroid in Graves' disease, or rheumatoid joints.

By the time Gus Nossal returned from his sabbatical in Paris, where he had gone to examine reports by Alain Bussard (22) that peritoneal cells could make multifunctional antibody against red cells, I was generating a lot of in vitro culture data. Some months later, when Erwin Diener left to head a new immunology department in Edmonton, Canada, Gus decided that I could keep the two technicians in Erwin's charge, which enabled me to carry on with a wider range of projects and not confront the classic PhD student's dilemma of too many ideas for the time and limited resources.

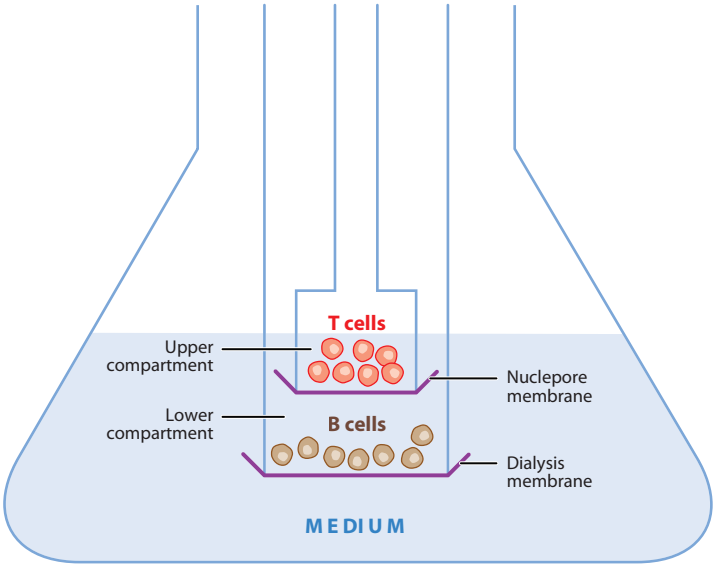


Figure 2

Double-chamber cultures, formed by concentric glass tubes, suspended in a reservoir of medium. T cells were placed in the upper compartment and B cell–containing populations in the lower compartment. Adapted from Reference 15.

Gus's decision had some interesting implications, and it was not popular with other staff. Still a PhD student, I was heading a little group working in immune tissue culture. In the methodological aspects, Alan Harris, who had trained with Renato Dulbecco in culturing tumor cells at the Salk Institute, was very helpful, querying the methods we used with spleen cells, compared with his own work with cancer cells. Gus offered me new students, and I had the opportunity to initiate John Schrader into the intricacies of immune cell culture. It was a challenge, from which both John and I escaped unscathed (23).

Hermann Wagner was the first of a group of talented young German medical scientists who came to WEHI to train, followed by Martin Rollinghof and Harold von Boehmer. Hermann had worked in complement and was intrigued

by immune cell killing; he wanted to develop an in vitro system for generating cytotoxic T cells (CTL). We set about doing this using the Marbrook-Diener culture system; the experiments succeeded and resulted in an in vitro generation of CTL. Hermann developed this research path enthusiastically over the ensuing years (24). Wunderlich and Canty at NIH had previously generated similar results using the Mishell-Dutton system (25).

Taking part in the collaborative atmosphere at WEHI was an incredible learning experience. Gus had a saying that I paraphrase: "Not publishing your data is a luxury few can afford." I took that to heart and suspect few PhD students have published more from their thesis time than I did, owing to the multiple collaborations evaluating immune responses in vitro. Many of these papers were written late at night, fuelled by coffee and the music of the Rolling Stones.

MOVING TO LONDON

Gus was a friend and contemporary of most senior immunologists, so contemplating a postdoctoral position in the laboratory of another famous scientist was a realistic possibility for me. High on my list were Gerald Edelman's lab in New York and Avrion Mitchison's lab in London. Because of funding problems in Australia (regular occurrences in all laboratories), Gus decided to delegate to me attendance at a small, intimate immunology conference at Brook Lodge, a retreat in Kalamazoo, Michigan, owned by the Upjohn Company and used for conferences. I was very privileged to be able to take part; so many luminaries whose papers I had read, such as Baruj Benacerraf, James Gowans, Fritz Bach, and Mel Cohn, were present. On the way, I visited Avrion (Av) Mitchison and Gerald Edelman and compared which lab might be more suitable for me. Av was a most charming host, inviting me to stay in his house and give a talk to his colleagues at the National Institute of Medical Research (NIMR) at Mill Hill, where there was a wonderful intellectual and friendly atmosphere (e.g., 26, 27). So I decided to join Av's group, but not at NIMR, but rather at the new Imperial Cancer Research Fund (ICRF) Immunology Unit he was starting at University College. Talented young colleagues there from the beginning, Marty Raff, Mel Greaves, and Nancy Hogg, were soon joined by Peter Beverley, Reg Gorczynski, Robert Tigelar, and Geoff Shellam, with Liz Simpson a frequent visitor. Subsequently, Mike Owen, Benny Chain, and Mary Collins joined us. All have gone on to major scientific careers and contributions.

It was an exciting place to be, rich in intellectual resources and modern scientific equipment, with Av's friendship with the Herzenbergs securing him one of the very first fluorescence-activated cell sorters (FACS) (28). Scientific visitors abounded, to give seminars in the crowded, small seminar room in the Department of Zoology, with Av, lying back in the front row with his feet up, eyes almost closed, as they gave their seminar, but very much awake, as question time revealed. Memorable was a young Peter Doherty coming to tell the world of his very surprising findings with Rolf Zinkernagel (29) of the genetic restrictions in CTL activation and of the various interpretations they were exploring.

Moving to London had amazing advantages. No longer was there the tyranny of distance and isolation that have so preoccupied legions of Australian scientists to this day, and the United States, where almost half of science was being performed, was now only between 7 and 10 hours away and could be visited for a few days. The exhausting 24-hour trips from Melbourne to the scientific centers of Europe or the United States were no longer necessary. For example, there was no need to choose which of two major conferences to attend: Travel time and exhaustion level were no longer deciding factors. I was invited to many conferences, and I went to a lot. There was more money for research than in Australia, and more scientists were available for the skilled, labor-intensive immunology research. Added to these career benefits were the numerous cultural attractions of London. Av readily organized an

appointment for me to the research staff of ICRF, and my planned return to Australia after a two-year fellowship was postponed, indefinitely as it turned out.

I did make trips back with my family so that my children could visit their grandparents. On one of these trips, I bought my first souvenir of tribal art from Australia, an aboriginal bark painting, from a Melbourne dealer. A love of tribal art, encountered first in Fiji on the way to London, has remained with me. My office is now crowded with African and New Guinea masks and sculptures. I believe (an untested hypothesis) that being surrounded by original creations fosters creativity!

THE EARLY MAGICAL DAYS OF CYTOKINES

In the early 1970s, I developed a multichambered culture flask to study whether cell contact was necessary, and this generated a keen interest in intercellular mediators (15). The technology back then for identifying these mediators was far inferior to today's. And so although important biological activities were present in supernatants and were given names to reflect that [e.g., osteoclast-activating factor, macrophage-activating factor, T cell growth factor, B cell–stimulating factor (30–33)], their molecular identity remained a mystery. An approximate molecular weight was as far as the effort got, as the potency of cytokines meant that there was very little protein. The number of potential mediators described was growing fast, and to try to make sense of this, Joe Oppenheim and others initiated the first of a series of conferences that grew into the Cytokine Conferences. The first was near NIH in 1977, and the second was at Ermatigen, Switzerland, in 1979.

The first conference focused on clarifying the problems of the field; by the second, attendees suggested that some bioactivities might coexist within the same or related molecular species. We agreed upon the nomenclature interleukin, with IL-1 potentially encompassing lymphocyte-activating factor, osteoclast-activating factor, and endogenous pyrogen, all based chiefly on similar molecular weight and origin, and IL-2 being T cell growth factor. A consensus paper was published from this conference chiefly reflecting input from Kendall Smith and Joe Oppenheim (34–37).

But the real turning point in this field came with the use of new technology, driven by perceived clinical need. Molecular biology techniques had invaded immunology in the mid- to late 1970s and had instigated real progress, such as cloning of antibody genes (38) and clarifying the generation of antibody diversity. Interferons (anti-viral mediators) were considered to be potential cancer cures (39), and so a lot of work was emerging in the late 1970s to scale up their production. By 1979 (40), Tada Taniguchi had cloned the first type I interferon (IFN) cDNA, closely followed by David Goeddel and Sidney Pestka (41) and Shigekazu Nagata and Charles Weissman (42). Cloning of interferon was closely followed by the cloning of IFN-γ and other important interleukins, IL-1 (43), IL-2 (44), IL-4 (45), IL-6, etc., in the 1980s.

By 1984, the cloning of tumor necrosis factor (TNF) and lymphotoxin were reported, first presented at a Cytokine Conference at Schloss Elmau by David Goeddel. By this time, I had started collaborating with Ravinder (known usually as Tiny) Maini (see **Figure 3**) on the role of cytokines in the pathogenesis of rheumatoid arthritis (RA). The properties of pure TNF described by Goeddel (46) were highly suggestive of those relevant to RA. The molecular biologists were providing new tools for elucidating the properties and function of cytokines, and, with that, many aspects of pathology and medicine were to change dramatically.

CYTOKINES AND UNCOVERING MOLECULAR CLUES TO AUTOIMMUNITY

Science progresses by testing new ideas or hypotheses. In the early 1980s, there was increasing realization that in various autoimmune disease sites, there was upregulation of major histocompatibility complex (MHC)

Key collaborators

Ravinder (Tiny) Maini

Fionula Brennan

Mike Shepard

Jim Woody

Figure 3

Important collaborators throughout my career: Ravinder Maini, Fionula Brennan, Mike Shepard, Jim Woody.

expression, especially of MHC class II. This was found in rheumatoid synovium by Klareskog and Wigzell (21) in Sweden and Janossy (47) in London, and in endocrine autoimmune tissue, thyroid, and pancreas by Franco Bottazzo and Ricardo Pujol-Borrell (20). Franco came to see me to discuss whether this upregulated class II expression had any immunological meaning. To someone like me, having worked for many years on mechanisms of T cell activation, including antigen presentation, the answer was obvious.

But it seemed a bit too obvious. Could upregulation of antigen presentation, induced presumably by environmental events, be sufficient to trigger autoimmunity in genetically susceptible individuals? The latter point is critical because of the importance of genetics, especially MHC, in regulation of the immune response, as Hugh McDevitt (48) first showed. Experiments in both mice and humans had demonstrated that autoantigen-reactive T cells were present in nondiseased individuals. How might upregulated MHC be connected to autoimmunity? There seemed to be a clear scenario, based on Steeg and Oppenheim's (49) finding that IFN-γ upregulated MHC class II expression. The pathway might run as follows: Local tissue infection, perhaps viral, or other local damage would release cytokines and autoantigens, activating local cells to augment their MHC class II and antigen-presenting function. The cytokines and autoantigens would then be able to activate nontolerant autoantigen-reactive T cells, which in turn would activate effector cells, B cells to generate autoantibody, and macrophages to produce cytokines and other mediators, together causing more tissue damage, cytokine release, and so the vicious cycle of an ongoing disease. With the possibility of abnormal suppressor or regulatory T cells, I could thus envision the pathogenesis of a chronic disease.

I defined this scenario in early 1983, while staying with my family in a holiday home we had just bought in Begur, on the Costa Brava. While there, I had the time and freedom to think critically and write this hypothesis. With coauthors who had generated the relevant data, it was published in the *Lancet* as an untested hypothesis, a format that now seems very antiquated (50). When was the last major concept published without any supporting data? Of course, that would raise the possibility that a rival group could scoop yours by generating the experimental evidence. Nevertheless, 25 years later, this hypothesis is still a reasonable approximation, and rereading it is not at all embarrassing.

Testing this new concept experimentally was exciting. The necessary techniques were already available: Cell culture methods had progressed rapidly, and the understanding based on Kendall Smith's work on T cell growth factor (35) and on antigen presentation permitted rapid evaluation of the new hypothesis. Very important in testing this hypothesis were two young colleagues, Jonathan Lamb (now a professor in Edinburgh, then a postdoc who had greatly improved human T cell cloning techniques while in Jim Woody's lab) (51) and Marco Londei (an enthusiastic and bright medical graduate new to the lab but keen to make his mark in medical research). We passed the first test swiftly: adherent thyroid cells, a population including many epithelial cells and some antigen-presenting cells, were able to restimulate, after influenza peptide incubation, MHC-compatible influenza-specific T cell clones (52). Other tests took a little longer. Cloning T cells from Graves' disease samples was a challenge that Marco relished; he cultured the lymphocytic infiltrate first with IL-2 to select for in vivo–activated T cells and then cloned them (53). Seeking cells that were restimulated by autologous adherent thyroid cells but not allogeneic thyroid epithelial cells was accomplished, and he obtained wonderful pictures of T cells stretched and adherent to epithelium, as well as more quantitative proliferative data. Subsequent work in collaboration with thyroid experts Basil Rapoport and Sandy McLachlan (54) and postdocs Sonia Quaratino and Colin Dayan identified the diversity of autoantigens recognized, thyroid-stimulating hormone receptor, thyroglobulin, and, most often, thyroid peroxidase (55). We analyzed the cytokines able to upregulate epithelial cell MHC expression and showed that IFN-γ and TNF were both important, varying with cell type. This work was driven by Ricardo Pujol-Borrell and my PhD student Ian Todd (56).

So the cellular basis, the outline of the hypothesis, had been rapidly tested and substantiated between 1983 to 1986. Scientific interest in this concept was high; transgenesis was becoming an effective research tool, and so transgenic mice overproducing IFN-γ in the islet cells of the pancreas, driven by the insulin promoter, were generated by Nora Sarvetnick at Genentech, and these mice duly developed autoimmune diabetes (57). But of course many questions remained unsolved. Were the epithelial cells really the antigen-presenting cells initiating disease? This went against the dogma. Did the epithelial cells have a role in disease maintenance or in recruiting immune cells? To evaluate the medical significance properly, we needed to identify the intercellular mediators involved, cytokines or others. But this was not possible with the operative samples of thyroid that could be obtained after the disease was quiescent enough to permit safe surgery. Furthermore, thyroid diseases have never been seen as major unmet needs because their treatments, while imperfect, have been good enough for a long time.

WONDERFUL COLLABORATIONS AND FRIENDS

The ethos of effective collaboration—the pooling of diverse skills and resources to permit a more effective attack on a major challenge—had pervaded WEHI. Having learned the power of effective collaboration, I joined forces with Franco Bottazzo on my first serious venture into autoimmunity, which made considerable progress. But personality differences limited this joint venture. I sought a more important autoimmune disease, like thyroid with a local site of disease that could be immunologically studied, and RA was an obvious choice. Nathan Zvaifler, a leading U.S. rheumatologist, had come in the late 1970s to do a sabbatical with Av Mitchison, and I had the good fortune to be charged with looking after him. I suspect we both learned a lot from each other: Nat gained some insights into the ever increasing complexity of immunology, and I discovered that RA was a major immunological disease with many aspects not yet understood, which made it an important unmet need. When I subsequently rang to ask him who in London was

the best person to work with in this field, he unhesitatingly said Ravinder (Tiny) Maini at the Kennedy Institute of Rheumatology (KIR), whom I duly rang. His enthusiasm for a potential new collaboration was evident, and he was in my office accompanied by Lindsay, his laboratory technician, within two days. That was the start of a truly wonderful collaboration and friendship, which has transformed our careers and enabled the very difficult task of translating laboratory science into effective therapy. It began with a detour, however. Systemic lupus erythematosus (SLE) was the major disease being studied by Maini's group at the time, and so I explored whether the techniques used in Graves' disease might be useful in studying SLE.

With Tiny's encouragement, I rapidly got involved in arthritis research, successfully applied for an Arthritis Research Campaign grant, and then was invited to see the new research center being built on the site of the Charing Cross Hospital, which had plenty of lab space compared with my base at the ICRF Tumor Immunology Unit at University College. Professors from Charing Cross and Westminster Medical School had gotten together to raise support for this new center, and Mary Glen-Haig, then chief administrator of the hospital, had found donors. Her friend Sir William Shapland, chief executive of the building firm Bernard Sunley & Sons, was the chairman of the Sunley Trust and of the group planning this research development, the Charing Cross Sunley Research Center. Because my work on autoimmunity was progressing well and because, being an optimist, I could envisage that it would eventually be tested in patients, I no longer felt it was appropriate to be personally supported by a cancer research organization, even if it was very rich and broad-minded, as the ICRF led by Walter Bodmer and Mike Crumpton was.

Moving to an empty building (even if only four to five miles away and with only a few key staff) to build up a team is traumatic and difficult. One always underestimates the financial and equipment needs. We had ambitious plans to discover the key molecular mediators in active RA synovium and develop new therapies aimed at interfering with them. I do not know the percentage of teams with such ambitious therapeutic goals that actually succeed, but it is certainly not high.

We had certain key assets, including two leaders, one at the laboratory end and the other at the clinical end (Tiny), but we also had an appreciable overlap of understanding. Another asset was excellent team spirit, facilitated by the involvement of several fellows and students who had previously worked with one or the other of us. Working in the rapidly developing cytokine field was enthralling, but its clinical importance had yet to be established or understood. It was a wonderful challenge, which was chiefly supported financially by a variety of research charities. The Arthritis Research Campaign was the major one; it has had a large, long-term investment in KIR since its beginning in the 1960s and in my work at the Sunley Research Center from 1985, before I joined KIR (the Sunley Research Center became incorporated into the Kennedy Institute in 1992). This long-term funding made such risky research much more possible than funding on three-year grants that The Wellcome Trust, Nuffield Foundation, and Sunley Trust all contributed. Most importantly for the long-term challenge of this work was that Tiny and I were good friends. It is not clear that we would have worked so closely and effectively for over 20 years, overcoming various problems, had we not had the trust in each other that friendship brings.

Joining me initially at the Sunley from University College were several key postdocs, including Marco Londei, whose work on Graves' disease I mentioned above, and the late Glenn Buchan, from Otago, New Zealand. Glenn had begun successfully to use molecular biological techniques to study cytokine and cytokine receptor expression in synovium and was involved in refining them to permit use with small human diseased tissue samples (58, 59). Regrettably, he died early in 2008 from cancer. Very important for the extensive grant writing that was needed was that my secretarial assistant Philippa Wells also decided to move with me.

In retrospect, certain experimental choices were vital for understanding the role of cytokines in RA. First, we needed to focus on which mediators were actively synthesized at the site of disease, using mRNA analysis through the cloning of cytokine genes. Second, for studying regulation of cytokine production in rheumatoid synovium, we did not use the classical techniques of culturing and passaging rheumatoid synovial cells, a complex mixture of cells, until only adherent synovial fibroblast-like cells are left. This technique made no sense to me, as 80–90% of the initial synovium, the hemopoietic-derived immune cells, were thus discarded, and, of course, after several passages, the environment of the remaining cells was very distinct from the initial environment (60). Hence, we used short-term cultures of the entire cell mixture in synovium (3–7 days maximum) to study synovial cytokine regulation. These experiments were carried out chiefly by postdoc Fionula Brennan (58, 61), now a professor at KIR. From her work emerged the first evidence that TNF might be a therapeutic target. She demonstrated that in rheumatoid but not osteoarthritic mixed synovial cell cultures, anti-TNF antibodies dramatically reduced the production of IL-1 (61). Subsequently, this was extended to anti-TNF downregulating a range of other proinflammatory cytokines, GM-CSF, IL-6, and IL-8, which was all encouraging news (62–64). But it was worrying that anti-TNF also reduced the anti-inflammatory mediators, such as IL-10, IL-1 receptor antagonist, and soluble TNF receptors. This work is summarized by the TNF-dependent cytokine network concept, illustrated in **Figure 4**, which has proved a useful approximation to the truth.

Richard Williams tested in animal models the hypothesis that TNF was a therapeutic target, using the collagen-induced arthritis model that he had already established in Tiny's group (65). All animal models are imperfect; this one was less imperfect than others and was very useful in demonstrating the need for high concentrations of antibody for maximal efficacy. It also showed clearly, by immunohistology, that leukocyte infiltration was markedly

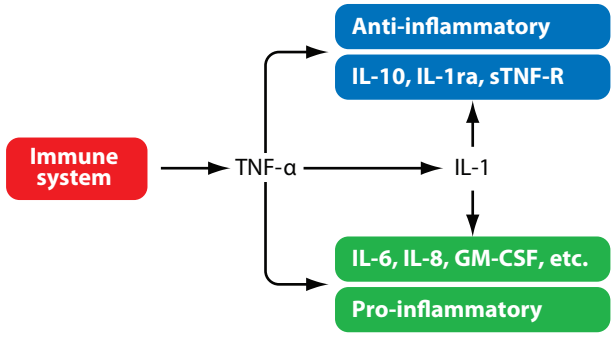


Figure 4

TNF-dependent cytokine cascade in rheumatoid arthritis (RA). This was an important component of the scientific rationale for anti-TNF therapy in RA.

reduced, and it demonstrated joint protection of both cartilage and bone. This joint protection was known in 1991 in the mouse but was not verified in humans with RA until 1999. In this work, we were greatly assisted by Robert Schreiber, who donated his anti-mouse TNF monoclonal antibody, unique at the time. He generously provided this hamster antibody in large amounts, without which we could not have done the work. It is of interest that coming from other vantage points, two other groups, of Thorbecke (66) and Piguet (67), concurrently demonstrated the benefit of TNF blockade in mouse models of RA.

Tiny Maini had started his research career at KIR with Dudley Dumonde. He had been involved in studying lymphocyte mitogenic factors in the late 1960s and had coined the term lymphokines (68) well before there was technology for identifying such rare molecules. So he was very aware of the importance and potency of such mediators, and exploring the role of cytokines in RA was thus for him also a natural progression. While I encouraged my colleagues to improve the sensitivity of techniques needed to quantitate cytokines, Tiny had the unenviable task of masterminding the collection of abundant samples of rheumatoid synovium needed and characterizing their clinical phenotype and biological marker profile. To this day, I am puzzled that it is so difficult to get the majority of surgeons to take the extra 1–2 minutes to ensure that the correct tissue

is collected in a sterile manner into the appropriate bottle of medium. The rare exceptions appear to be surgeons who understand the research process and the dependence of medical progress, indeed of all progress, on research.

The cytokine field was revolutionized by the molecular biology skills of the biotech industry. Scientists at a number of companies, e.g., David Goeddel, Pat Gray, and Axel Ullrich at Genentech and Craig Smith at Immunex, cloned cytokine genes, expressed the proteins, and generated antibodies. I made contacts with these companies, and they donated antibodies and cytokine reagents without the awful material transfer agreements that plague collaborative research today. Particularly important were antibodies to TNF, produced at Genentech, which had first cloned it. At Genentech the extramural program manager then was Michael Shepard, who took a great interest in our work and helped us considerably to unravel the role of TNF in RA. He supplied all the cytokines, cDNAs, and antibodies to TNF and to LTα/TNF-β. After a few years, he returned to his own cancer research career, which was very productive, and he has found fame as the initiator, scientific champion, program manager, and developer of the anti-HER-2 antibody, trastuzumab, better known as Herceptin®, which has saved many thousands of lives of patients with breast cancer (69, 70). A personal reminiscence: When David Goeddel first presented his group's work on cloned TNF in 1984 at Schloss Elmau, I went to talk to him and was told that as the TNF project was a collaboration with a European company only they could supply European labs with TNF reagents. However, the European company was worried that my hypothesis that cytokines such as TNF might be involved in pathogenesis of disease would negatively influence the development of what they had wanted, which was a cytokine cancer cure. So they did not want to help me, but fortunately the Genentech research-driven culture did.

James Woody, a U.S. Navy medically trained researcher, was a protégé of Ken Sell who had benefited from getting his PhD in the UK in three years, much quicker than was possible in the United States. Jim was rising rapidly in the U.S. Navy Medical Research Command, and Ken Sell, his chief, sent him to Av Mitchison in London to obtain his PhD. Somehow he ended up under my supervision. And being a very bright, diligent, well-organized scientist, he duly finished in the minimum time, often bringing his children to the lab at weekends in order to do so. He returned to Bethesda, jumping from being a PhD student to running a big laboratory for the U.S. Navy. From there, his career progressed in leaps and bounds, emulating his mentor Ken Sell to reach the top of the U.S. Navy Medical Research. While he was at the Navy, we kept in close touch, and he knew of our burgeoning work on TNF. The U.S. Navy funded some of this research. By the time Jim had finished his 20 years in the Navy, in 1991, and was considering pharmaceutical and biotech opportunities, he was aware that we were close to defining TNF-α as a therapeutic target in RA. So it was very pleasing that he opted to join an emerging biotech company, Centocor, a pioneer in developing the monoclonal antibody field. John Ghrayeb and his team at Centocor had grafted the human constant region to antibody variable genes from a murine anti-TNF hybridoma generated in Jan Vilcek's laboratory (71), in response to Tony Cerami's powerful arguments that blocking TNF-α might save thousands from death in sepsis (72). So in early 1991, before Jim had officially started, I visited Centocor and presented our work leading up to the definition of TNF-α as a therapeutic target in RA. It received a warm reception, especially from Hubert Schoemaker, the chairman/CEO. Some of the company scientists were more skeptical, especially their only rheumatologist who somehow knew that anti-CD4 antibody would be much more effective for RA therapy than blocking TNF. Centocor was focused on sepsis, and in Europe, their IgM monoclonal antibody to LPS had been approved, on scant data. In the United States, it had not yet been approved, and so an interesting deal was set up, basically that I (and my colleagues) would help them to define

the mechanism of action of their LPS antibody and how it protected despite its low affinity, and they would help us with testing our TNF therapeutic target. Our academic-led project was not a normal clinical program. Jim Woody as chief scientist ran it instead of the clinical group, with the help of a number of Centocor staff, including Hanny Bijl, Dick McCluskey, Carrie Wagner, and Tom Schaible. The chimeric monoclonal antibody cA2 had already been administered in high dose to several dozen patients with sepsis. It failed to correct the septic shock, but importantly they did not get worse. This reassured us that its use in RA trials would not lead to overwhelming infections.

THE EXCITEMENT OF CLINICAL TRIALS

The first trial was an open study with no placebo controls, nonblinded owing to the unknown risks of blocking TNF in rheumatoid patients. Ten patients were initially planned to be treated with the high dose found necessary in mice. When the striking results of the first patients were disclosed to the company, Centocor did not know exactly what to do, so it asked us to treat 10 more. Of course with 20 patients responding, it was easier to draw conclusions and publish than with only 10 (73).

The results had matched or even exceeded our expectations. Over the slow (3 h) infusion of the antibody, many of the patients commented that they were already feeling better, less tired. Over the next day or two, reductions in stiffness and pain were noted. Large effusions in knee joints rapidly diminished. There had been concerns that blocking TNF, a host defense molecule, might promote infection, and so we had taken the precaution of starting the infusions slowly, with just one patient first, treating them as inpatients, and we had our own nurse spend the night in their room in order to treat possible problems as rapidly and effectively as possible.

It was a very thrilling time. All the patients we treated improved dramatically, despite having had long-standing active RA refractory to current treatment. The first two to three months were especially interesting because we did not know how long the benefit would last. Patients returned to their normal activities, holidayed, played golf, etc., and were really happy. They thought the improvement might be long lasting. But it was not to be. There were 12 to 18 weeks of marked benefit before relapse. There were no cures, but nevertheless there had been major improvement and a clear pointer for the future. I helped coorganize a conference in Arad, Israel, near the Dead Sea, with my friend David Naor. It was there, in mid-September 1992, that Maini first presented the dramatic results of the first clinical trial. There were scientists from other companies, from Immunex, Genentech, Roche, etc., and the disclosure, probably premature owing to our naiveté, started the race toward the clinic, as these companies had already produced TNF inhibitors for use in sepsis, based on Tony Cerami's work (74).

To establish what might happen with longer-term anti-TNF treatment, we sought ethical permission to retreat some of the patients from this first trial after they had relapsed. Eight of 20 were retreated, up to three times. In each case, there was reintroduction of significant clinical and biochemical benefit (e.g., reduction in C-reactive protein), suggesting that if TNF was blocked, other cytokines did not rapidly take over to drive the cytokine network (75).

But this initial experiment was not a formal proof. There had been no controls or randomization, much needed in clinical trials with potentially high placebo responses. To do that, a formal double-blind (patients and clinicians), randomized, placebo-controlled trial was performed. Three European rheumatology friends, Joachim Kalden of Erlangen, Ferdinand Breedveld of Leiden, and Josef Smolen of Vienna, joined in with Tiny and me. There were issues to resolve, such as what to use as placebo. We chose human albumin to avoid immunizing patients to mouse antibodies, and the primary end point was limited to four weeks for ethical considerations and to reduce drop-outs in the placebo-controlled trial. Again, the results were

very clear and convincing. Both the high dose used previously and a tenth of the dose worked well, but the placebo infusion did not (76). We collected large samples—400 ml of blood from these patients—to do a very detailed analysis of the post-treatment events. The mechanism-of-action studies were very informative, most importantly because they confirmed that there was a very rapid diminution in other proinflammatory cytokines, for example IL-6. The reduction to baseline of a downstream cytokine in a few hours is evidence of a direct effect of anti-TNF (76, 77), validating the TNF-dependent cytokine cascade concept.

The mechanism-of-action studies were performed in considerable detail, possible because it was an academic-led study, with the blood samples under our control. Few other clinical programs to date have been analyzed in such detail. We also looked at cellular changes in the blood: They were less informative than we had hoped but showed a rapid reduction in circulating neutrophils and monocytes. More interesting was a rapid increase in lymphocyte counts, which tend to be low in active RA, with more activated cells in the blood. The rapidity of the change suggested that there was a change of trafficking, probably an exit of T lymphocytes from the joints. We were able to explore the hematology in more detail, and important pathogenic clues emerged. There was an increase in the hemoglobin concentration (usually low) in the patients within the four weeks of the trial. The high platelet counts in RA and high fibrinogen, both potentially linked to accelerated atherosclerosis, tended to normalize (78). This was a clue that the abnormal cardiovascular outcomes in RA might be improved, but it took a long time for other groups to establish this, in large post-registration registries in the UK that Alan Silman, David Isenberg, and their colleagues have ably run (79, 80), as well as those in Sweden (81, 82) and other countries.

Cell infiltration in the joints is a hallmark of chronic RA. The reduction in joint swelling suggested that fewer cells persisted after therapy. Biopsy studies clearly showed that to be the case, with reductions in lymphocyte and macrophage numbers and a thinner lining layer. How did this occur? Attempts to show increased apoptosis were not successful, but we did find reductions in markers of cell recruitment. Thus, endothelial-specific E-selectin was reduced, both as detected in synovium by immunohistology and in serial serum samples as soluble form. Also reduced were intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, both involved in cell recruitment to tissues. The more quantitative serum soluble adhesion molecule assays were consistent with the less quantifiable histologic reductions. In a similar way, we also found that numerous chemokines are reduced in the synovium as well as in the blood (83, 84). Markers of tissue destruction were also diminished. Serum matrix metalloproteinase precursors are elevated in active RA and were reduced after anti-TNF therapy (85). Of course, serum assays fail to demonstrate what is active in the joint, but they do reflect the biosynthesis during active disease.

Rheumatoid joints are very cellular and have often been described as resembling tumors. To sustain this augmented mass, new vessels are needed, and so angiogenesis is readily apparent. Ewa Paleolog and colleagues observed that angiogenic factors are also augmented in RA, and it was of great interest that the most potent of these, vascular endothelial growth factor (VEGF) (86), was rapidly but partly diminished after anti-TNF therapy. However, it took a lot of subsequent immunohistological analysis by Peter Taylor and colleagues to demonstrate a reduction in blood vessels (87).

It was remarkable how much molecular work could be performed from one small clinical trial: We obtained many longitudinal samples but still had many unanswered questions. The results all pointed toward normalization of the pathological processes, and while they did not show that TNF causes arthritis, they showed that TNF is a very important driver of active disease. In the mouse, the elegant work of Kollias and his colleagues has shown that transgenic mice overexpressing TNF does cause an erosive polyarthritis, even in mice lacking T and

B cells (88, 89). We had learned much about disease mechanisms from the detailed work on the first placebo-controlled trial. But there was a subsequent disaster. The freezer holding most of the samples from the next trial, a longer phase II, over six months, defrosted. The consternation, angst, and frustration were awful; it was physically painful to think of the major scientific opportunities lost. In the phase III trial, Centocor controlled the samples to speed up the trial process and the hoped-for drug approval; hence, far fewer samples were collected, and the opportunity of investigating biological markers in greater detail was lost.

By 1993, there was clear evidence of clinical benefit, even in the most disease-active patients, but no cures. Retreatment was successful, but we were concerned about immunogenicity of this antibody, which, while chimerized (2 faces, 3/4 human constant region), still had the mouse variable regions that were likely to be immunogenic (71). Whether it was a drug that could be used for long-term therapy was unclear, so research was planned to learn how to augment the benefit and reduce immunogenicity. As is the case for all major diseases (e.g., cancer, hypertension), combination treatment is necessary to optimize clinical benefit. So we used the mouse model of collagen-induced arthritis to pilot potential approaches to augment benefit.

It was not difficult to produce anti-TNF nonresponder mice. We needed to reduce the treatment dose to 50 μg twice per week instead of the efficacious 300 μg (65). In this model, using suboptimal doses of anti-TNF antibody, a range of additional T cell–directed therapies were tested, and cyclosporine, anti-CD4, and CTLA4-Ig (90–92) were all effective, suggesting that there was enhancement of the clinical benefit if T cell function was also reduced. In these experiments, there was synergy, as the effects of anti-CD4, cyclosporine, and CTLA4-Ig as monotherapy after disease onset were rather modest, if present at all (91). So from these animal model studies, especially the anti-CD4 experiment, the clinical trial design evolved in which patients with an inadequate response to methotrexate (MTX) were treated with various concentrations of anti-TNF (by that time known as cA2, later infliximab, later Remicade®), in order to augment their response.

MTX is effective in a significant proportion of rheumatoid patients, as demonstrated and championed by Michael Weinblatt in Boston (93, 94). In the 1990s, its impact was growing, and it was becoming recognized as the most effective disease-modifying antirheumatic drug. As it would not be possible to use two unlicensed drugs together (anti-CD4 is unlicensed), Tiny Maini and I chose to use MTX, which had been reported, among its legion of effects, to inhibit T cell function, promote apoptosis, and reduce IFN-γ production (95, 96), effects that resembled those of anti-CD4. Patients who had an inadequate response to MTX were abundant, and so our trial was designed to fill an important clinical need. But an issue was the risk, especially of infection, in the combination. So a very low dose of MTX was chosen, 7.5 mg/week. However, at this time Centocor was struggling financially, and the long-term clinical trial we had envisaged was shortened to 24 weeks, 12 weeks on therapy and 12 weeks further follow-up. Nevertheless, the results were very interesting and have been influential. Lower-dose cA2, 1 mg/kg at weeks 0, 2, 4, 8, and 12, was effective, with about 25–30% of patients showing 50% benefit [using the American College of Rheumatology (ACR) 50 criteria], only up to week 4 if used alone. But with low-dose MTX, there was clear synergy, with 60–70% ACR 50 up to week 24 (97, 98). The results using higher doses of cA2, 3 mg/kg and 10 mg/kg, also showed the added benefit of the addition of cA2 to MTX. It is now the combination most extensively used in routine practice, with about 70% of patients given the existing three anti-TNF drugs also being given MTX because of the increased efficacy (99, 100). After Tiny had first presented the exciting initial clinical results to Centocor management, Jim Woody made cA2 available to Sander Van Deventer, an enterprising gastroenterologist in Amsterdam, who successfully treated a Crohn's disease

patient with fistulas (101). Centocor then prioritized resources to Crohn's clinical trials, to our dismay. This became the first approved indication for cA2, now known as infliximab or Remicade®.

Despite its clinical priorities and cash limitations, Centocor agreed to fund an important imaging mechanism-of-action trial to investigate leukocyte trafficking to joints before and after anti-TNF therapy. This was performed by Peter Taylor, who now ably leads the Kennedy clinical trials group, together with A.M. Peters, a leukocyte imaging expert at Hammersmith Hospital. This trial demonstrated that anti-TNF reduces leukocyte traffic to joints (84). This was an important clinical trial because reduced recruitment of inflammatory cells to disease sites probably accounts for the ability of anti-TNF to ameliorate so many diseases.

With the success of the phase II trial in which MTX was supplemented with cA2, the multinational phase III was planned and eventually successfully executed with Peter Lipsky (a friend from the 1970s from his time as a postdoc at NIH with Alan Rosenthal) as the U.S. trial leader and Tiny as the European leader. The complexities and grind of phase III trials made this a nonexciting and stressful, though necessary, experience compared with the earlier trials. But through the whole process, working with Tiny was an enjoyable, educational experience, as we blended his rheumatological and other clinical skills with my immunology and cytokinology and entered fields new to us, where success rates had been dauntingly low.

PROS AND CONS OF WORKING WITH INDUSTRY

Academia and industry often do not mix well. Having studied medicine, I was interested in the practical application of immunological research. My industrial interactions started while I was still a postdoc. James Howard worked on immunity to polysaccharides (102), a field analogous to one of my research topics, immunity to polymerized flagellin (103). Both were repeated polymers that induced thymus-independent antibody responses. He invited me to consult with his group at Wellcome Research Labs, a small pharmaceutical company, known locally at the time as University of Beckenham for its academic bent. It had recruited Nobel Prize winners John Vane and James Black to run its research. This was a wonderful start to the pros. There was much to learn, and one was paid extra, which is very appreciated early in one's career, with growing children and increasing bills to pay!

My second exposure was with ICI Pharmaceuticals, the precursor of Zeneca, now AstraZeneca, when my PhD student Eric Culbert joined them: I have had a number of long-term consulting relationships, helping friends and colleagues. For example, I consulted with ed David Webb, first at Syntex, then OSI, Syrrx, and now Celgene; Michael Moore; Jim Woody at Centocor, then Roche. I advised Michael Shepard, first at Genentech, on the Herceptin project, then adenoviral gene therapy while he was at Canji, then targeted cancer therapy at Newbiotics, and now at Receptor Biologix. These long-term relationships were in many ways very educational. Thus, our work on adenoviral inhibitors for studying cytokine and other intracellular signaling pathways developed from an awareness of the utility of adenoviruses developed while helping Canji.

But the work with Centocor was on quite a different scale, and despite its many frustrations at times, it was very beneficial and helped drive anti-TNF therapy forward. Like all the best interactions, mutual benefit is essential. In 1992, Centocor was a rapidly growing biotech company that thought it was going to be the one to capitalize on treating sepsis with monoclonal antibodies. It had an IgM (Centoxin) anti-LPS monoclonal antibody approved on limited data in Europe and was looking forward to new data and approval in the United States. When Jim Woody joined them as chief scientist, he was keen that we help them to fill a gap, to understand how Centoxin mediated its benefit (104). As this research would involve our field of expertise, cytokines, it was logical. In return, it would be easier for him to encourage his

colleagues to invest in and become interested in our untested and not yet accepted concept of TNF as the therapeutic target for RA. My colleague Peter Katsikis (105) duly found a novel mechanism by which complement may assist in endotoxin clearance, and Jim succeeded in getting Centocor to provide financial and antibody resources to test anti-TNF therapy. The success of this interaction led to a promising novel therapy for a major unmet need and, in my opinion, eventually saved Centocor from extinction, as the endotoxin project failed and Centocor's share price crashed in 1992.

Centocor had charismatic leaders Hubert Schoemaker and Michael Wall, and it tenaciously survived its problems with sepsis therapy and went on to be acquired for almost $5 billion by Johnson & Johnson in 1999. The early trials with Jim Woody at the helm and Harlan Weissman, a key player, were run harmoniously, as were the first placebo-controlled trial and the six-month phase II trial. But then as the likelihood increased that clinical success would translate into commercial success, the management of the trials became focused on commercial speed of completion, and thus were run very differently.

By this time, Jim Woody had left Centocor to become president of Roche in Palo Alto, California, and Hubert Schoemaker had been weakened by a brain tumor and its very aggressive therapy. We were left to manage the Centocor-KIR relationship with no internal champions. It was not congenial: Agreements and promises were reneged; stress ensued. Matters important to academics, such as publication and presentation rights, were challenged and arbitrarily overturned; issues of the nationality of presenters of key data were raised. It is almost unbelievable that it was felt in the late 1990s that an American was a more credible presenter of data than a European. Agreements about authorship were subsequently ignored by the company. Even worse is what happened after the drug was approved and started to sell and the other two TNF inhibitors also were sold. The respect scientists have for each other's discoveries is often not shared by industry and its lawyers. In Bob Dylan's words, "money doesn't talk, it swears."

I regret that anti-TNF is yet another of the British inventions that was not commercialized in Britain, but rather in the United States. This happened despite very extensive discussion in the late 1980s/early 1990s with the UK's leading monoclonal antibody company. But this company missed the golden opportunity taken up by Centocor and its U.S. rivals, Immunex and Abbott, that has resulted in approximately $11 billion in sales of anti-TNFs in 2007.

But overall, I am an enthusiastic supporter of working with the biotech and pharmaceutical industries. Many of the skills needed to get new treatments into the patient population are possessed by industry: medicinal chemistry, pharmacology, and especially the financial resources for major clinical trials. These are complementary with academia, and if these complementary skills were harnessed more appropriately, society would undoubtedly benefit. My actions with many ongoing industrial relationships probably speak louder than any words on this topic, and the practical outcome of the interaction between academia and industry is that there are now no more RA patients in wheelchairs.

PROMOTING TRANSLATIONAL RESEARCH

What is translational research? There is no agreed definition, and that is part of the problem, but by conventional usage it is research designed to further human health: to bridge new discoveries in basic research and the applied research in clinical trials of therapeutic products.

A major challenge in research has always been which experiment to perform, among the countless possibilities. Sir Peter Medawar has elegantly expounded on this, and "The Art of the Soluble" is not an exact science (106). How high to aim at any one time can result in major disappointment if the effort fails, but if it succeeds, wonderful gains can be achieved. So every scientist expresses their unique personality in their choice of projects and approach. I was acutely aware that despite the major

advances in cellular and molecular understanding of immunology, its key practical applications, vaccines, date back 200 years to the time before immunology was a major science. Why had there been so little progress, despite stellar advances such as the discovery of monoclonal antibodies by Kohler & Milstein (107)?

Organizing Intense Translational Scientific Meetings

Communication remains a problem in all complex organizations and societies, and scientific meetings have an important role in science. I first became interested in organizing scientific meetings in 1983, wanting to promote the relatively new techniques of T cell cloning and their potential for helping unravel disease pathogenesis. This meeting took place in 1984 (108), after I had worked out how to raise funds for these small, intense, focused meetings. Critical to the venture was James Woody, when he was in the U.S. Navy, who convinced the Navy to support meetings. As he has progressed in his career, via Centocor and Roche, he has for many years been the major funder of many scientific meetings and made the meeting organization much less stressful for me. A key benefit of organizing these meetings has been that my colleagues and I learned, made contacts and friends, and initiated important collaborations, with the help of discussions on the lawns and at the bar during these conferences, chiefly held at Trinity College, Oxford (109, 110).

Many experiments were hatched at these meetings, which have been variously named T Cell Activation in Health and Disease; T Cells and Cytokines in Health and Disease; From Laboratory to the Clinic. I have held 20 of these meetings, coorganized with Andrew McMichael, a fellow at Trinity, again with the skilled enthusiasm of my long-term personal assistant, Philippa Wells, who has now capably helped me for 27 years, all financed through friends and acquaintances in the biotech and pharmaceutical industries.

But while translational research is now a hugely popular theme, from NIH with Dr. Zerhouni's roadmap to the newly revamped Medical Research Council under Sir Leszek Borysiewicz, it is still very ill defined, and many fail to understand its basic principles. Translation is the apex of the pyramid, an activity suitable for only a minority of projects. It depends first on excellent quality of science, but second on science that reveals an important rate-limiting step in the complex biology that occurs in vivo. Not only that, the complex biology must be relevant to humans and their diseases, and not just to mice. I paraphrase the late Judah Folkman, who said in the late 1990s, when there was hype about blocking angiogenesis curing cancer: If you are a mouse with cancer, we can help you, but if you are human, it may take another 20 years. Humans differ from mice, most obviously in their longevity. With longevity there is a need for more robustly regulated biological systems for the many years before reproduction. So while mouse systems need to function for 10–12 weeks and are subject to Darwinian evolutionary selection pressure only for this period, for humans the selection process is more than 100 times longer, for over 20 years. We expect differences in complexity to emerge, so mice are not likely always to be an accurate model for human pathophysiology.

It is thus puzzling and a continual challenge that many medical journals still fail to appropriately prioritize and encourage research performed with rare human disease material, often because all the controls cannot be performed as well as in mice. The latter is the current bandwagon, as much progress is based on elegant genetically engineered experiments. But many failures in successful translation of laboratory research into disease relevance are likely due to technical issues such as the overuse of reductionist systems, interspecies differences, or the use of transformed cell lines, with their many mutations, as surrogates for normal human cells.

Directing the Kennedy Institute of Rheumatology

Directing KIR has been a major focus of my activity for the past six years since Tiny Maini retired as director. The KIR is the largest research institute dedicated to rheumatology and has been supported effectively by renewable long-term (five-year) major grants from the Arthritis Research Campaign in the UK covering 40–50% of the total budget and increasingly by the Kennedy Institute of Rheumatology Trust. The director's role is to evolve strategy, recruit the best talent, provide them with the best resources, and let them get on with their research. Leading by example works better for most scientists than direction. KIR's focus is translational research, from laboratory to clinic and back again, and it has a wide range of expertise from molecular science, proteomics, molecular modeling, etc., through cytokine biology, immunology, inflammation, matrix biology, and signaling to clinical research and trials. KIR has long been a global resource for research and training in rheumatology and related disciplines. My challenge is to leave it in an even better state than I found it when my friend Tiny transferred it to my care.

It has been a privilege to work there long term with many talented colleagues, almost 25 years with Sir Ravinder Maini, 20 years with Fionula Brennan, almost that long with Brian Foxwell, and 10 years with the burgeoning osteoarthritis team leaders, Jeremy Saklatvala and Hideaki Nagase. The team spirit, mutual support, enthusiasm, and intellectual challenges make it a pleasure. Interacting with the Faculty of Medicine, Imperial College, which the KIR joined in 2000, opened up new avenues and access to many multidisciplinary colleagues in other branches of medicine, engineering, chemistry, etc. Taking part in the creation of the UK's first Academic Health Sciences Centre led by Stephen Smith, from the merger of Hammersmith, Charing Cross, and St. Mary's hospitals, has been educational. The administrative issues at KIR, in a constantly changing scientific environment, are less entertaining than the science, but the prospect of helping to deliver the fruits of research more effectively for our patients makes it worthwhile.

CONCLUSIONS

Maintaining Life/Work Balance

Science is fun, and should be fun. It is the ultimate experience in solving puzzles, puzzles that no one has previously solved, and you are even paid to solve them. If you are not able to enjoy the excitement and thrill of science, to enjoy the roller-coaster ride, and to shrug off the inevitable frustrations of failed experiments, malfunctioning equipment and colleagues, and rejected papers and grants, then a career in science will be more pain than pleasure and perhaps is not a wise choice for you. But with the fun and excitement comes the inevitable huge work load, and maintaining a life/work balance is a challenge that few can successfully manage. For those working in Europe, at least there is the hallowed tradition that long holidays are beneficial, but my U.S. colleagues seem to take far fewer holidays. They spend more time in the lab, but does that add up to greater productivity? Having enjoyed outdoor activities and sports while growing up in Australia, I know long holidays provide not only an opportunity to enjoy family, friends, and the splendor of our planet, but also time to think creatively and strategically. Some of my best ideas emerged thousands of miles from the laboratory. A challenge for all scientists is to optimize their productivity; my warning is that more time in the lab might not be the best way. Eventually, we all learn that time is life's most precious commodity.

Do We Value Practical Research Contributions?

All of life is influenced by fad and fashion, and science is no exception. The term "blue sky research" (for pure basic research) clearly implies basic research's desirability, whereas in contrast applied research implies sweat rather than

inspiration. But is this really true? I could make an argument for the reverse. Thus, much basic research is not inspired, and, in the past quarter of a century, it has evolved from many projects following a preset pattern chiefly purifying a protein, to cloning the mRNA for a gene, to making transgenics and knockouts. Clearly, as techniques become better defined, much diligence is necessary, but how much really new, creative, inspirational research takes place per project? I suspect that what really matters in research is its quality and imagination, and both are always needed for the best, pure, basic, blue sky, applied, or translational research to succeed at the highest level.

My own experience is that society does indeed value research contributions of a practical or applied nature. If it is practical or applied, the effects of the research are easier to measure than they are with basic research. However, there appears to be greater delay before success in practical research is recognized, which is inevitably frustrating. The frustration stems in part from the delay of being recognized or rewarded only by objective concrete delivery, rather than by subjective promise or potential. In due course my work with my many collaborators has resulted in much personal recognition, including election to the National Academies of Science in the UK and Australia, honors such as the European Inventor of the Year award in the Lifetime achievement category, the Curtin Medal of Australian National University, and the award, together with Ravinder Maini, of a series of prestigious international prizes for medical research such as the Crafoord Prize of the Royal Swedish Academy of Science and the Albert Lasker Award for Clinical Medical Research. But the greatest reward of practical contributions, added to the respect of one's peers, is the heartwarming acknowledgment by patients of the positive impact on their lives. It is an unexpected pleasure.

Prospects for the Future

It is entertaining but challenging to review what one has helped to achieve and then predict what might happen next. What I have helped to achieve, first, is that a cytokine, TNF, is now recognized as a very good therapeutic target for a cluster of chronic inflammatory diseases, including RA, juvenile RA, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, psoriasis, and ulcerative colitis (111). This raises the question of how important TNF is as a fire alarm for noxious signals bringing in the fire fighters, leukocytes, and whether most conditions currently treated by corticosteroids might be treatable by anti-TNF.

Second, my colleagues and I have helped to demonstrate that biological therapeutics, that is, monoclonal antibodies and antibody-like fusion proteins, can be used for chronic diseases in the long term, now very long term (up to 10 years and running). This has inevitably influenced the pharmaceutical industry, and now a very significant percentage of new therapeutics entering trials are of this type. There are, of course, major benefits. An important one is that biologics, with a large surface of interaction with their target, are more specific and selective than the small molecular, organic chemicals traditionally favored by the pharmaceutical industry. Hence, their side effects are more predictable because they are mechanism related. The unfortunate TeGenero disaster, in which an activating anti-CD28 monoclonal antibody was used to try to stimulate regulatory T cells, is worth noting. Some believe it was unexpected or unpredictable. However, most human immunologists like myself, who are aware of the variable toxicity of OKT3, an anti-CD3 antibody, which polyclonally activates T cells, believe it was extremely predictable (112). This disaster and the ensuing publicity have markedly influenced clinical trial capacity in the UK.

There have been a number of subsequent successes for monoclonal antibodies and cytokine blockade. Anti-CD20 antibody, developed for lymphoma based on Ron Levy's work, has been very successful (113) and was introduced to rheumatology by Jo Edwards (114). IL-1 blockade with IL-1 receptor antagonist has been approved but, because it

has been less effective than TNF blockade, has not been widely used in RA (115). It has been very useful in, for example, Muckle-Wells syndrome. There is clear effectiveness of blocking the IL-6 receptor with an antibody developed by Tadamitsu Kishimoto (116, 117), and there is great anticipation for the utility of blocking RANK ligand for bone disorders (118).

So an interesting possibility emerges. Are all diseases and unmet medical needs treatable by cytokine blockade? As an optimist I believe that will be close to the truth. The 100 or so cytokines (a term that I use to encompass interleukins, growth factors, IFNs, chemokines, members of the TNF family, etc.) are involved in all key biological processes, for example cell proliferation, cell motility, inflammation, immunology, angiogenesis, fibrosis, etc. Hence, all diseases involve alterations in cytokine expression, and many are upregulated. These are potential therapeutic targets. My colleagues and I are pursuing important new therapeutic endeavors that might be treatable by cytokine blockade. For example, with Claudia Monaco, we are studying treatment of atherosclerosis; with Mervyn Maze, we are studying post-operative cognitive dysfunction; and with Tracy Hussell, Brian Foxwell, and Kendall Smith, we are studying acute respiratory distress induced by avian flu. Only time will tell if these endeavors will succeed, but inevitably the field of cytokine blockade or anti-cytokine medicine will flourish in many more directions.

Why should one bother to read semihistorical personal reviews? I am not sure, but there may be lessons for the less experienced. If so, one is that we now have wonderful technologies for permitting scientific progress in the field of disease pathogenesis and therapy. These can unravel molecular mechanisms of diseases and permit the discovery, design, and development of new treatments that impact millions of lives. But this will not work for most projects, as most projects and hypotheses fail. But it will succeed for some. So there are enormous opportunities remaining to use science for the benefit of human health and welfare. But the hurdles are tough and the risks high. I encourage as many clinicians as possible to spend the time and training, as I did, to merge both science and medicine, as there is a critical shortage of individuals, for example physician-scientists, who can synthesize these components to bring the translational discoveries to patients. Perhaps a summary of an exciting adventure that has benefited many patients might encourage and challenge you to venture into that arena and see what you might achieve.

DISCLOSURE STATEMENT

Over the years, I have interacted extensively with companies and so have been a paid consultant or scientific advisory board member to many companies involved in arthritis and cytokine work: Amgen, AstraZeneca, Abbott, Centocor, Glaxo, Celgene, Immunex, Merck, Roche, Wyeth, Novo Nordisk, Schering Plough, Boehringer-Ingelheim, Synovis Ltd., Xenova plc, Hydra Biosciences, Receptor BioLogix, Inc., Nuon Therapeutics, Canji, Inc., Trillium Therapeutics, Inc., Sandoz (now Novartis), Alza, Inc., Almirall Prodesfarma, Ferring AS, and Calyx Therapeutics. I or close colleagues have received grants in the past three years from Roche, Wyeth, Novo Nordisk, Celgene, Nuon Therapeutics, and Receptor Biologix, Inc. I have patents in the anti-TNF therapy field and many others (I was European Inventor of the Year in 2007). I have significant financial holdings in Johnson & Johnson and Schering Plough.

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