Section I: Brief Scientific Biography

I, as many, liked the world of ideas from when I was small. I read some philosophical books in my early teens, mainly by Bertrand Russell, but came to the belief that studying physics could provide better opportunities to appreciate and come close to beauty in nature.

I studied physics whilst at Gonville and Caius, College, Cambridge, from 1961-1964, as an undergraduate. I found the experience frustrating; there was not enough time for me to understand and appreciate the vast amount of material. I felt I could not do justice to my aspirations or the subject.

I knew I wanted to be in a field where I could try to make some significant conceptual contributions and hopefully do some related experiments. I had little confidence I could achieve this in physics. I was very interested in theory. The particular field did not seem so important to me, but rather the approach one took in an attempt to make progress. I was very fortunate. My brother, Mark, who had trained as an organic chemist, was doing his PhD under the guidance of Sydney Brenner and Francis Crick in the area of protein synthesis and the genetic code, in the Laboratory of Molecular Biology, in Cambridge, England. I was accepted to do graduate studies at the same laboratory with John Kendrew, who already had his Nobel Prize for the structure of myoglobin. My graduate studies were to be in the field of protein Xray crystallography.

To have been at The Laboratory of Molecular Biology at this time was a unique privilege and allowed one to witness history. I taught myself some genetics and took an interest in many things. Crick and Brenner were interested, among very many things, in the origin of the genes coding for antibody chains. I thought the model by Brenner and Milstein on this topic, published in Nature, implausible, and brought my considerations to Sydney Brenner, who was very open to discussion. There were several hours of discussion every Saturday over coffee in the second floor laboratory “kitchens”, on scientific or other matters, with Sydney almost always present. This was the time of the acridine mutant work using T4 phage that gave rise to the experiments on the general nature of the genetic code, of experiments on co-linearity of gene and polypeptide, of elucidation of the genetic code and of the proposal for the Wobble Hypothesis and of allostery.

We had visitors I am unlikely to forget: Jacques Monod, Seymour Benzer, Frank Stahl, Jim Gowans, and Jim Watson, for example.

One day, I was doing an experiment and wearing plastic gloves to protect myself against a nasty chemical, bromo-acetic acid. I felt a throb in a finger as I fell asleep that night. The next morning one fingertip was quite white and without feeling. It was clear that a glove had leaked. The finger-tip was in time surgically removed and skin grafted

to the raw end of what remained of my finger. However, the first morning after the accident, I saw a sharp line begin to develop that demarked the healthy from the unhealthy tissue. I interpreted this as reflecting immunological self-nonself discrimination. I was already somewhat interested in immunology, as a result of my interaction with Sydney Brenner. Cesar Milstein had suggested I read some Burnet, but after reading a bit, I gave up as I felt much of what Burnet said was a bit wild. I started speculating about various aspects of immunology as described in the third section of this profile. I talked to Crick about several ideas. He suggested I see Avrion Mitchison, for a potential postdoc position, which I did. However, Crick suggested, as I talked to him more about my ideas, that I would find Mel Cohn of the Salk Institute a very compatible individual to do a postdoc with, given our common love of speculation. Crick was a Visiting Fellow at the Salk Institute at that time. A series of circumstances led me to realize during my PhD studies, and then find evidence in the literature that I could use to support the idea, that antigen, interacting with a single lymphocyte, inactivates it, and that activation requires the antigen-mediated cooperation of lymphocytes. I realized that these rules provided a potential explanation of immunological self-nonself discrimination, as I describe later (Section III).

Mel Cohn went to a Brooke Lodge meeting only a few (two or three?) weeks after I arrived at the Salk Institute, California. He talked about the ideas on the induction and paralysis of immune responses, as the processes of activation and inactivation of lymphocytes were then called. We decided that we had better publish our ideas quickly. I am not sure this was the wisest decision, but we thought a formal presentation was better than leaving the point of view we had open to misconception. I wrote a covering letter explaining the situation to Nature. I recall that Nature accepted the publication one day after they received it. Never anything remotely like this has ever happened again in my life! There were some aspects of this first exposition that troubled at least me. Resolution of these aspects led us to formulate the Two Signal Model of Lymphocyte Activation, published in Science, in 1970.

To have been at the Salk Institute at this time was also a great privilege. Neurophysiologists from Harvard came every summer and gave mind bending lectures. Various visiting Fellows also came a few weeks every year, including Crick and Monod. I got to know Jacques Monod quite well, as he was always interested in new ideas in immunology. Monod and I also had some considerable, good-natured, philosophical arguments. The diversity of lectures at the Salk Institute was probably unique. Some of the most interesting and speculative lectures were on the origin of life, because one of the Fellows, Leslie Orgel, was a prominent individual in this field. Some of the most beautiful were on phage lambda. Dulbecco, whose laboratory was devoted to understanding the biology of the oncogenic viruses, polyoma and SV40, felt that lambda's tricks might be relevant to understanding the tricks oncogenic viruses got up to. We had seminars on philosophical subjects and on animal behavior, and an attempted lecture on race and IQ. This lecture never took off the ground as it was disrupted. These were Vietnam days.

The individuals in Mel's laboratory for the most part had two properties in common: most (though not all) worked on immunological subjects and, of all that I knew, none had a background in immunology, until Michael Bevan joined the laboratory, shortly before I left. The real sign of experimental sophistication in those days was if you could inject mice intravenously! I remember one individual with a background in phage genetics becoming very distraught on finding a mouse that he had killed had two spleen. He was distraught, as one cannot do genetics with dead mice (anyway, not then). He wasn't really all that relieved when someone pointed out to him that he had been removing kidneys. It is unbelievable, in retrospect, how we lived. There was very little pressure from Mel to do experiments if you were a postdoc, and in some sense rather little guidance, unless you consider a lot of enthusiasm and more suggestions about what was important than anyone could digest. I also think Mel was fortunate in some of the young people he had. David Schubert, Sidhartha Sarkar, Alan Harris, Alan Sher, Bob Coffman, Jim Watson, Chuck Kimmel, Alan Harris, Jim Watson, Mario Caesari, and Martin Weigert were all contemporaries of mine, and I overlapped with Michael Bevan a bit at the end of my fellowship.

Besides my involvement in The Two Signal Model of Lymphocyte Activation, I did theoretical work on autoimmunity and immune class regulation. Bob Coffman was one of the individuals who kindly read and gave me comments on the manuscript describing my theory of immune class regulation. I did not publish anything from my experimental work. This was not for want of trying. I was inexperienced and Mel's lab was in its early days. I was also a bit foolish. I developed one series of experiments testing whether a circumstance, under which lymphocytes should be inactivated according to our two signal ideas, did indeed lead to inactivation, and got the kind of result I anticipated. However, the results were not as clear-cut as I had expected. I took this to mean that my understanding of the phenomenon was inadequate, and so I thought I should not publish. Similar but even less clear work along the same lines was later published and was regarded as pretty significant. I have learnt from this to better accept that my understanding is never complete, and to therefore be both more realistic and humble.

Another aspect of our intellectual life was its freedom, unbelievable in this modern day of urgency and competitiveness, illustrated by two endeavors. Many people became interested in neurophysiology, with the summer lectures by the gifted Harvard professors. It was hard not to be fascinated. I spent some time studying David Marr's Theory of the Cerebellum, and gave a few talks on it, as understanding it required considerable effort. Sidhartha Sarkar and I decided to try to learn some neurophysiology together. We got a self-teaching book on neuroanatomy, and a brain we were going to dissect. The point of these tasks we had set ourselves, after a few attempts at a bit of cramming, became questionable. We decided we would only need to know neuroanatomy, once we had some interesting ideas, that would propel our learning all that stuff. We never of course did develop those interesting ideas and therefore never learnt a significant amount of neuroanatomy.

The second endeavor had more long-term consequences. A group of us felt we should be studying a subject that might be more relevant to the relief of human suffering than basic immunology. We decided to study together schistosomiasis, a disease that caused much suffering world-wide. Donato Cioli, Paul Knopf, Alan Sher and I were all in this group, and all took the plunge of going into immunoparasitology, except me. I decided to keep my interest in the subject, but to remain in basic immunology for the present. I hoped that my contributions in basic science might also help in some way to relieve human suffering in the longer term.

I returned to Cambridge, England, to the Babraham Institute of Animal Physiology, in 1972. I had established myself as a theoretical immunologist whilst in Cohn's lab. I knew hardly any immunologists in England, so my options were limited, but I did know Cambridge well, and some immunologists there. After rather less than a year, I saw an advert for a job at The Australian National University (ANU), for a person with research interests in an area suspiciously close to mine. This was too good to be true. Moreover, the Head of the Department was a well-known immunologist, Gordon Ada, whom I had met on his way through the Salk Institute. I applied, with some anticipation. However, the job had been ear-marked for Chris Parish. Chris had actually done a lot of very interesting experiments, and these had in part stimulated my Theory of Immune Class Regulation. No wonder the job description made me think the fit with my interests was too good to be true!

Although I did not get the job advertised, Gordon Ada made other arrangements so that I could go to his department at the ANU. I was very interested in doing so, because I knew that the department really was a laboratory of professional microbiologists and immunologists, not exactly the description one would provide for Mel's lab, and I really wanted to develop my experimental skills. Moreover, I had some clear ideas on what type of experimental approach should be explored to test some of my proposals underlying immune class regulation.

Chris had a graduate student, Ian Ramshaw, who got interested in the theory. I think his other experiments had not been going so well. We discussed different approaches as to how to test various ideas. It was a very nice journey we took together. I remember Ian coming up with a paper describing an experimental system that he thought might be useful. It was. At one stage, I decided to do a job tour that took some weeks. When I returned, I found Ian had made tremendous progress. We felt we had really accomplished something significant. The experiments seemed to fit the predictions beautifully.

The John Curtin School for Medical Research, of the Australian National University, was another special place, set up, as was The Salk Institute, with a generosity of spirit. In our Department of Microbiology, Alastair Cunningham, who kindly gave me some space in his laboratory, was exploring with Linda Pilarski whether genes,

encoding immunoglobulin chains, hypermutate following antigen stimulation of the B cell carrying them. A few months before I arrived, Peter Doherty and Rolf Zinkernagel, working together in Bob Blanden's lab with the type of systems Bob had established, had made the first observations demonstrating the MHC-restriction of cytotoxic lymphocytes, for which they received the Nobel prize some years later. Here too there was an unusual freedom for intellectual exploration. It is this kind of enthusiastic environment that leads to significant and bold ideas. Alastair Cunningham had been Kevin Lafferty's first graduate student. Kevin was interested in transplantation and overcoming the transplantation barrier. This had led him and Alastair to try to understand some phenomena related to the activation of T cells. They related their work to our Two Signal Model of Lymphocyte Activation. So I came into a very interesting and very interested environment.

Australia was incredibly beautiful, and I very much liked the people. I was asked very informally if I might like to be considered for a better kind of job at the ANU. I decided that Australia was too far away from family. I often subsequently wondered whether I had been too hasty.

I had made a tour whilst at ANU looking for possibilities for my next career step. Linda Pilarski, who had done her postdoc with Alastair Cunningham, was at the University of Alberta, in Edmonton, Canada, so I visited the Department of Immunology there. The Head was Erwin Diener, who had also been in Australia at the Walter Eliza Hall Institute. I decided to go there.

I arrived at the University of Alberta in 1978, about nine years after my PhD in protein Xray crystallography. I had done some publishable and published experimental work whilst at the ANU, but I wished to develop myself further in this respect. I was successful in this whilst at the University of Alberta. Both Ian Ramshaw and I had decided that the next critical step would be to obtain an in vitro system in which antigen could induce primary cell-mediated (DTH) and humoral responses. In this way, we could examine what circumstance favored the generation of one response over the other. No one had managed to generate primary cell-mediated responses in vitro, with the exception of those to MHC antigens, which we felt were a special case. We kept in frequent touch. We used somewhat different approaches. Ian developed his system much faster than I did. He refrained from submitting his work until I had caught up, which took quite some time. Who under today's pressures could and would show such kindness and generosity?

I judged myself to be successful in doing significant experiments, and so felt I could now give students guidance. I agreed to take on my first graduate student, Jane Tucker. Also, I had help in the laboratory from someone I came to greatly respect, Mohamed Dhalla. After some years, Tim Mosmann joined the department. We were

both part of a small MRC group, that included Bhagi Singh. I also met Calliopi Havele, also an immunologist, who became my spouse.

In 1987, I moved to the Department of Microbiology and Immunology at the University of Saskatchewan. One advantage of the move was that the Department of Immunology at the University of Alberta had no microbiologists, in contrast to the Department at Saskatoon, and I really think this affects the way people think. I got a small grant shortly after arriving. This was given to new Faculty, on application, to start up a new area of investigation. I knew what to do. I had wanted to work on infection and immunity, in the mouse model of human cutaneous leishmaniasis, for some time. However, I regarded this as too risky an undertaking if using funds from my own MRC grant, obtained to study basic regulation of the immune response. Juthika Menon did her PhD developing the low dose vaccination strategy in this model.

We established, during the 1990s to early 2000s, a number of different experimental systems, investigating issues relevant to preventing and treating a certain class of infectious diseases. This class of diseases is defined by the ability of a strong cell-mediated response, in contrast to antibody, to contain the pathogen, and includes the leishmaniases, tuberculosis and AIDS. Cell-mediated immunity is also recognized as being uniquely effective against most cancers. The ability to develop new areas of investigation was only possible because of support in the laboratory from Goujian Wei, whose efforts, under my guidance, have opened up one new experimental system after another. This has allowed me to provide guidance to more graduate students. In addition, Juthika and I started working on human disease, mainly on tuberculosis and visceral leishmaniasis. We developed collaborative research in Ethiopia. The Saskatchewan Lung Association, formerly the Anti-Tuberculosis League, has supported our tuberculosis research in a particularly helpful way. We have studied immunity to intracellular pathogens that cause chronic disease, such as tuberculosis and the leishmaniases, in mouse models. The way these studies appear to shed light on human disease, and in some cases appear to dovetail with our human studies, is quite overwhelming. I hope I can contribute to and witness some real progress in prevention and treatment of these devastating tropical diseases. I felt and feel we understand enough about how the immune response is regulated that we are probably able to develop effective vaccination against TB and AIDS, and treat early stages of AIDS. It has been an incredible journey to go into new fields and obtain clear and striking results, consistent with one's expectations. However, my hope is somewhat dented by my losing all research funding from national granting agencies in the field of infection and immunity despite my greatest efforts.

I have had a strong interest in tumor immunology since the late 1960s. When funding for our research on immunity to infections became problematical, I tried to get money for doing studies on immunity against tumors. I had a simple hypothesis as to why the immune system often fails against cancer. We initiated these studies on tumor immunology in the early part of the 2000s. Duane Hamilton, then a graduate student,

asked me whether he could take on these studies. Guojian Wei had made some initial and very interesting findings. Duane's observations were very striking to both him and me and were anticipated on our hypothesis. They seem to open up new ways of guiding immunotherapy, if our mouse studies really reflect parallel situations to those that occur in human cancer. However, I have found it problematical to obtain funding for this research.

I had a heart attack in August 2006, when Calliopi Havele, my wife, and I were visiting our daughter's family and our first grandchild in Toronto. This attack led to emergency bypass surgery. I was 63. I had three days in intensive care, where the situation I faced, and perhaps the morphine I was given, made me reflect on my past, and how I might still achieve unfulfilled aspirations that I had formed in my youth. These aspirations were to interact and communicate with my fellow human beings about how one could understand and become close to, or feel to be part of, nature and face existential questions. I had envisaged that these aspirations could be realized through doing scientific research collectively. However, I had found that doing science, the way it is practiced today, is overall a lonely process. I was not realizing my dreams of joint discovery and pleasure, except with my students. A solution seemed obvious. I had always felt that basic ideas were communicable to most of my fellow human beings. I had never tried to join scientific bandwagons. It seemed to me that I might have a fresh start of realizing my aspirations by writing a book for the non-specialist on what scientific culture meant to me. I would illustrate this by the story of my involvement in immunology. I started writing this book within a couple of days of leaving hospital.

I describe in the second section of this profile the books I have written, and am writing now, and how I came to write them. I am 76 as I update this profile. I still have a lab with a wonderful, last PhD graduate student, Ghassan Al-Yassin. I still speculate on immunological topics. However, our experimental research over the last twelve or so years has been greatly hindered by my inability to get appropriate funding for either basic immunology, or in the related medical fields of autoimmunity, and immunity to pathogens and to tumors. I think there is a simple reason. I believe that the predominant conceptual frameworks, employed today to explore the most basic questions, are wrong and so misleading. It is not easy to get funded if one has frameworks contrary to those most widely held.

I have come to the belief that the culture supporting science has changed dramatically in the last 50 years. This change is driven in part by the information overload, and in part because most scientific careers have become lonely and competitive journeys, and so do not optimally contribute to a common realization. I believe both the influence of the information overload, and of the competitiveness that leads to individual rather than to collective journeys, are not only an impediment to progress, but undermine the spiritual inspiration that science can bring to our lives. Such influences have, to be overcome, to be diagnosed. The drive to write my third,

non-immunological book, in progress, is to explain the reasons for my thinking these influences are not inevitable and to explore how they can be transcended.

Lastly, the change in culture supporting immunological research perhaps reflects the change in the general culture, supporting science, in a particularly exaggerated form. Immunology has been, in the last three or four decades, one of the most rapidly expanding areas of biological research. Forty years ago, it was possible for most to have a rather general knowledge of the field. Now, few immunologists know much beyond the niche they are dedicated to exploring. I deeply believe progress in both basic immunology, and its impact on medicine, would be much greater if more attention was given to formulating what are the foundational questions of the field, and in fostering attempts to answer them. As indicated above, I believe some of the major frameworks commonly employed, in exploring what are recognized as critical questions, are incorrect. A knowledge of the history of some science illustrates how a change in basic frameworks can have unimaginable and unimagined effects.

I decided, about six years ago, to visit some universities and research institutions, to find colleagues with whom I could discuss the foundations of the field. I took part of my salary as a grant to support this journey. I did not give talks or accept payment for any expenses, as then I would have to give seminars and meet people on a formal basis. This would interfere with my primary aim. I wanted an opportunity to find individuals who were open to discussing foundational questions. It might seem strange to many readers that this was not possible with most researchers. However, I should point out in this context that many foundational issues were not being addressed by the immunological community, and would often involve a questioning of frameworks, commonly employed by the individuals in their own research. I had decided on a very gentle approach. I would not pursue a line of argument if I felt my colleague was at all defensive. I wanted to find individuals open to unfettered exploration of ideas.

I also went to scientific meetings with the same purpose in mind. I met two individuals through these journeys. Colin Anderson, also a Canadian, had a record of interest in basic questions and, though we had different perspectives, we found ourselves able to have meaningful discussions that influenced both our views. I met Alex Corthay, of Oslo University, at two meetings. Alex was very open to exploring ideas. The second time I met him was in Davos, Switzerland. My parents were Swiss, and I had a half ownership of a house, Casa Mispa, in the southern part of Switzerland. When I met Alex the second time, I expressed my regret I had a flight booked back to Canada; otherwise, I suggested, we could have met for a couple of days at Casa Mispa to discuss science. Alex responded by suggesting we arrange a workshop in the future. Casa Mispa is set in a truly wonderful and beautiful setting. I had always dreamt of having discussions there with colleagues. Alex proposed that Zlatko Dembic, also of Oslo University, would be a suitable and engaged participant, and agreed to approach him. I approached Colin Anderson, whom I had met on my visit to the University of Alberta. I also had been very impressed by Zoltan Nagy's book on a History of Modern

Immunology, and asked him whether he would like to attend, though I had never met him. Inger Oynebraten, Alex's friend and researcher in cancer immunology, and Calliopi Havele, my wife and a researcher in transplantation immunology, were members of our first, one-week Workshop on the Foundations of Immunology, held at Casa Mispa. We have now had five successive, annual workshops. We have made a couple of reports from the second annual meeting, and now are planning our sixth. Speaking personally, I have no doubt that these meetings have contributed to the development of my ideas on immunology. The kind of interaction we have is something that, it appears to me, to be both valuable but rare in the immunological community.

August, 2019

Section II: Books written and being written by Peter Bretscher

Rediscovering the Immune System as an Integrated Organ, FriesenPress, 2016

The Foundations of Immunology and Their Pertinence to Medicine, FriesenPress, 2016

On Science, Society and the Contemporary Condition, in preparation

How I came to write some books

I had a heart attack in 2006, when 63 years old. I consequently had a bypass operation and was in Intensive Care for three days. This traumatic experience, and close brush with death, led me, whilst recovering in the Intensive Care Unit, to assess whether I had realized my youthful aspirations. I had resolved, as a youngster when twelve, to attempt to become a scientist. I was very interested in theories. I was also interested in philosophy as a teenager, and felt the scientific attitude would naturally contribute to the further development of the culture of our society.

I looked back over my life as I came to in the Intensive Care Unit: my successes, my failures, and my life's path. I felt well about my modest contributions to science; the activity of doing science had more than fulfilled my anticipations of wonder, engagement, ecstasy, and satisfaction. I had also anticipated that doing science would, by the example, satisfy my aspirations to express how scientific attitudes can contribute to society. I recognized, whilst in the Intensive Care Unit, the illusion of this anticipation. Poignantly, I felt the scientific culture had changed dramatically over the 40 years I had been scientifically engaged, and this culture was no longer as inspirational as it had been at the beginning of my involvement. The overall scientific attitude, as represented by contemporary science, could, I felt, no longer provide guidance as to how society should face existential questions. It was also my conviction that this lack of beneficial influence would have dire consequences. This conviction led to my resolve to write a book. Recognizing I had been given another chance, I would explain my perception of how an optimal scientific culture could contribute to how society faces today's existential questions.

I started writing within days of being discharged from hospital. As many first time authors, I tried to include all ideas and topics of interest in my first book. An editor, on

reading a draft manuscript eight years in the making, gave me this assessment. I immediately recognized the assessment to be insightful. I feel the need to first say a bit about my life to explain how I came to write books, and why I appreciated the editor's advice.

How I came to be interested in immunology

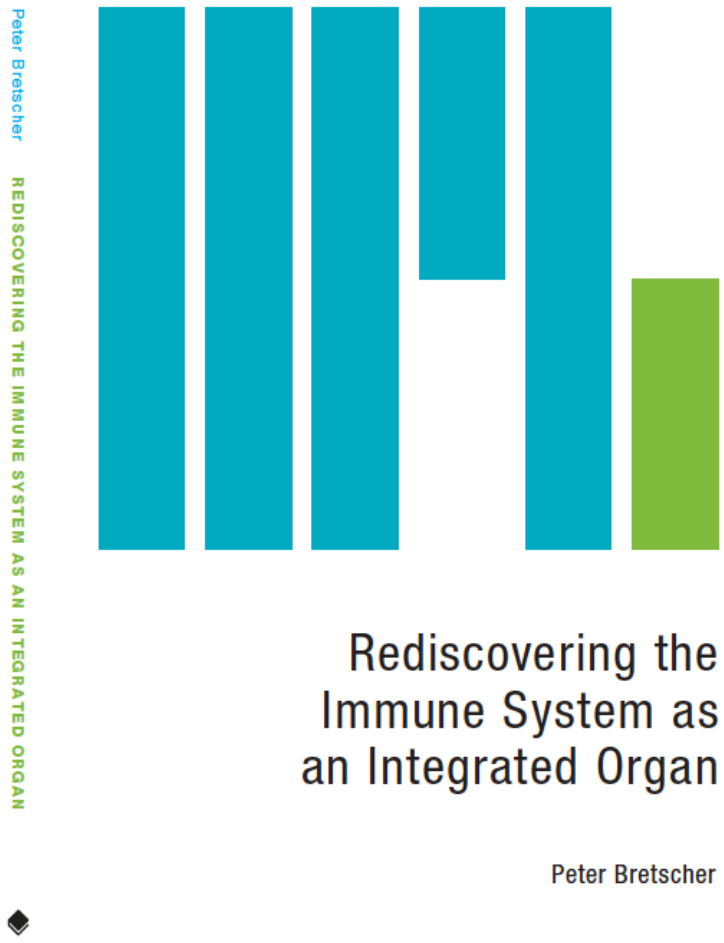
I studied physics as an undergraduate at Cambridge University, England. I sought, before graduating, to identify a rapidly developing science in which I might do graduate studies, to obtain a PhD, and to which I might hopefully contribute. I was lucky in being influenced to do, and being accepted into, graduate studies at the Cambridge Laboratory of Molecular Biology. My acceptance occurred in 1964, 11 years after Watson and Crick had proposed the structure of DNA. This lab was not that large at the time, though four of its senior members had recently been awarded the Nobel Prize, including Crick. Given my physics background, I was encouraged to do my graduate studies in the field of X-ray crystallography, employing X-rays to determine the three dimensional structure of proteins, under the supervision of John Kendrew. Kendrew had received the Nobel Prize for the determining the three dimensional structure of the first protein, namely "myoglobin".

I became fascinated, during my graduate studies, by immunology, the study of how the immune system works. The science of immunology is pertinent to preventing infectious diseases, by effective vaccination, besides being also pertinent to the prevention and curing of several other kinds of disease. My fascination took the form of trying to answer some of the most basic questions of the field. Crick had a notice board outside his office. He had pinned there an invitation, to those who wished to discuss science, to enter his office. I availed myself of this invitation a number of times to discuss ideas concerning various immunological issues. So began my life as an immunologist.

During the last 50 years, my students and I have been engaged both in making theories and in experimental studies in the field of immunology. The way I approach my research is influenced by the philosophical ideas I had developed as a teenager and undergraduate. Thus, in the first draft of my book, I gave an outline of our immunological research. This outline, I thought, could provide a case study for showing how the culture of science has changed over the last four decades, and provide a forum for a discussion of the effects of this change. I would try to explain how this change of culture undermined the effectiveness of the scientific approach, as now practiced.

My first book: Rediscovering the Immune System as an Integrated Organ

However, the constructive criticism of an editor, as already described, made me realize this was an ineffective way to proceed. I therefore decided to first write a book on how



broad considerations, in the context of experimental studies, could give rise to a coherent view as to how the immune system functions. It took me a couple of years, after receiving the editor's advice, to write this first book. I might add two significant features about the book and its realization. Firstly, I wanted to stress the value of certain foundational ideas as to how the immune system functions in a coherent fashion. We probably all know that scientists stress the importance of observation and of experiments to the success of their research, and I was very mindful in this book to explain not only foundational ideas, but the substantial observations supporting them, as well as the observations making alternative ideas seem less plausible. I anticipated that the book, for an outsider to the field of immunology, would be a pretty heavy read.

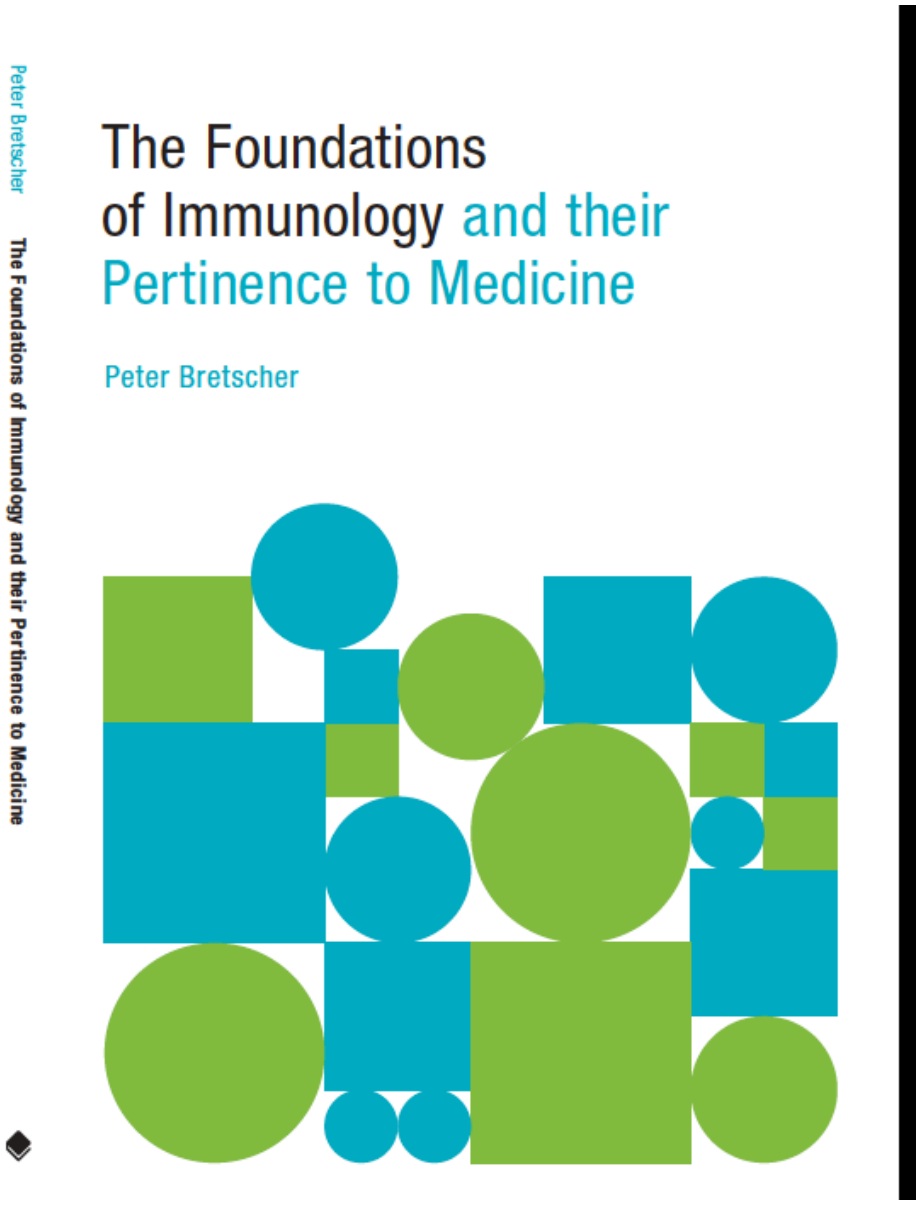
I initially submitted the draft to various University Presses, but had no takers. I therefore had to go with a non-university publisher, or find a firm that would allow me to self-publish. I had been in email contact with an immunologist, Zoltan Nagy, who had recently written a book, entitled "A History of Modern Immunology: the Pathway toward Understanding". Zoltan Nagy's book was produced by a commercial publisher. The cost

of his book was about five times the cost of what my book would cost if self-published. A main motivation in writing the book was to foster discussion, a process probably not optimally fostered by an expensive book. I therefore decided to self-publish with a Canadian firm, FriesenPress, in order to make the book broadly accessible. I outline below a couple of responses to this book.

My second book: The Foundations of Immunology and Their Pertinence to Medicine

I was quite surprised when, having seen my first book to press, I found myself in a virtual trance, committed to writing a second book. This book would stress the ideas I regarded as important to the field. I would employ observations to illustrate some ideas or to argue against the plausibility of others, but I would not attempt to observationally justify the foundational ideas I proposed. I was comfortable doing this, as my first book had provided such a justification. This justification gave me the freedom to primarily appeal to the reader's analytical abilities and imagination. Moreover, I could explain in the text how foundational ideas were pertinent to the prevention and treatment of many medical conditions. I found writing this text to be exhilarating. It was completed in three months. I felt I had achieved accessibility, brevity and clarity in my exposition.

The release of energy, to write the second book, initially took me by surprise. I had not even envisioned writing a second book on immunology. My energy arose from my intuition that the information overload of contemporary life has undermined imagination and faith in careful analysis, to the detriment of gaining real insights. My attempt to appeal to the reader's intuition and analytical ability, to overcome the weight of this overload, presented me with an inviting challenge.



I would like to explain a technical consideration concerning the production of my second book. Modern immunological texts are often between 500 to 1000 pages long, with fairly small type, and do not appear to me to optimally address the most foundational issues of the field. They actually often employ frameworks that I consider implausible, which makes the picture developed, of how the immune system functions, complicated. I find these modern texts to be overwhelming. I wanted the physical nature of my second book to reflect the idea that keeping to the basics allows the functioning of the immune system to be seen as simple and coherent. I told my Editor that I wanted the book to be printed in as large a type as possible. This would allow the reader, as they encountered the text, to feel they were making progress, as they turned the pages!

She suggested a fairly large print, and told me such a book would be about 180 pages. She also told me that, if the print were larger, the book would only be stocked in the children's section!

Comments on the books

I wrote these books with the hope they would substantially contribute to the field and be of interest to some of the general public. I made enquires concerning whether the books might be reviewed in various journals, but to no avail. This quite frankly surprised and somewhat shocked me. I submitted the first book to the New England Journal of Medicine, perhaps the premier medical journal. They were much politer than most. They explained to me that they no longer reviewed books. They had done surveys and found most of their readers were relatively uninterested in book reviews, and so had decided, as editorial policy, not to do reviews. Another journal gave me a similar reply. Most journals did not respond to my enquiry.

Reviews/Comments on Rediscovering the Immune System as an Integrated Organ

One colleague, to whom I sent a very late draft of the book, was both enthusiastic and generous. Ted Steele wrote: "Those who have followed Peter Bretscher's work these past 50 years will recognize this book for what is it – a rare achievement, a scientific masterpiece. It is a must read for all those Immunologists and Clinicians who want to find effective immunological cures for the many debilitating health issues that confront us. Peter Bretscher has produced a lucid and logical expression of the rules governing how the adaptive immune system responds to all foreign antigens, whether bacterial, viral or modifications of self, which emerge in cancer or autoimmune conditions. This book is a testimonial to Louis Pasteur's dictum that there is no applied science, just the application of basic science."

Dr Alex Corthay, Head, Tumor Immunology Unit, University of Oslo, wrote a review for the Scandinavian Journal of Immunology.

I quote part of the review below:

The immune system may seem incredibly complex. Researchers in immunology are amassing enormous amounts of detailed information without gaining proportional insights. Why might this be? So asks Peter Bretscher near the start of his book *Rediscovering the Immune System as an Integrated Organ*. He argues that contemporary immunology fails to provide understanding at the level of the system

because it is dominated by molecular and cellular considerations. He reminds us of a famous quotation: *Not everything that counts can be counted and not everything that can be counted counts*, before stating the ambitious aim of his book: to make plausible an integrated and readily accessible view of how the immune system functions. By Peter Bretscher. FriesenPress, 2016. 288 pp. ISBN: 978-1-4602-7406-4.

Peter Bretscher is a well-known immunologist who has been doing research for almost fifty years. His most famous contribution to the field is the two-signal model of lymphocyte activation, which he published in 1970, together with Melvin Cohn. This model, which provided an explanation for how self/non-self discrimination is realized, constitutes a pillar of modern immunology thinking. - - - - - Although very ambitious in its content, with both theoretical and applied immunology, this book is very didactic and well written. Results from key experiments are illustrated by simple figures which allow the reader to comprehend the original observations. Similarly, major theories are illustrated in a clear fashion. An extensive reference list invites to further reading. Each chapter is summarized by a brilliantly written synopsis. It is a must read for anyone interested in immunology, a classic book already.

Reviews/Comments on Foundations of Immunology and their Pertinence to Medicine

I sent a copy of a draft of this book to Dr Zoltan Nagy, immunologist and author of A History of Modern Immunology: A Pathway toward Understanding", even though I had not personally met him. Zoltan kindly offered to write a comment on the book for the back cover: "*Foundations*, despite its small size, is a monumental edifice of immunological thinking which could not have been written by anyone else but Peter Bretscher."

Rachel Jagareski reviewed the book for Foreward in February, 2017. She gave the book five stars (*****), and headed her review with:

From an innovative theorist and an elegant writer, this book is a valuable contribution to literature on medicine and disease

Excerpts from the review:

In The Foundations of Immunology and Their Pertinence to Medicine, research scientist Peter Bretscher summarizes historical discoveries and foundational concepts of immunology to encourage potential medical breakthroughs among non-specialists, clinicians, and "broadly educated" thinkers. The book presents overspecialization among scientists and medical professionals as a trend that limits the creative and

synergistic study of human illness. Bretscher sees many possibilities for treating and curing a variety of human ailments, from seasonal allergies to leishmaniasis to cancer, through an understanding of the ways immune system components variously attack pathogens and cells within the body to cause autoimmune diseases. Most of the book forwards a historical overview of immunology, from its beginnings in the eighteenth century with Edward Jenner's observations of smallpox immunity among cowpox survivors through to the very latest research into the molecular structure and function of cells within the immune system. The chapters gradually build up the layers of information needed to understand how lymphocytes and other cells are triggered into action to fight off infection—and conversely, why they don't in other circumstances.

Bretscher analyzes mountains of immunology literature and points out areas where further research could clarify previous findings or explain contradictory results. His is a thorough review that tosses out enough exciting ideas to occupy graduate students around the globe.- - - - - - The final chapters are the most exciting and reveal specific avenues for potential immunological treatment for a number of diseases. The author's analysis suggests that immune responses might be very specifically regulated to release certain antibodies, or to unleash one of the range of cell-mediated responses in specific situations, holding promise for many medical conditions. He envisions immunological scenarios allowing for the creation of vaccines against HIV and tuberculosis; targeted medical treatments for arresting multiple sclerosis and other autoimmune diseases; screening for susceptibility to certain kinds of cancers and inoculation to trigger immune protection; and protocols for protecting against the rejection of transplanted organs and tissue. Bretscher is an innovative theorist and an elegant writer, and this book is a valuable contribution to literature on medicine and disease. It provides ideas for further research into harnessing the body's highly individual immune response to vanquish ailments that have plagued humankind for centuries.

Dr Alex Corthay, Head, Tumor Immunology Unit, University of Oslo, also wrote a review of Foundations for the Scandinavian Journal of Immunology. I quote part of the review below:

We are in the era cursed with overspecialization. Charles Darwin's foundational *Origin of Species*, published in 1859, was accessible to the broadly educated individual. Starting with this statement, Peter Bretscher, in his new book, aims at explaining the foundational concepts of immunology to a broad public. His standpoint is that foundational ideas usually can be simply expressed. This book describes first how foundational concepts of immunology have evolved over the last two and a half centuries. The importance of self–non-self-discrimination by the immune system is acknowledged, and models for immunological tolerance are compared. A main focus concerns the understanding of immune class regulation. According to Bretscher's Threshold Hypothesis, a low antigen dose or low degree of foreignness results in Th1

immunity whereas a high antigen dose or high degree of foreignness induces Th2 responses. A novel vaccination strategy is proposed with an ultra-low number of live, attenuated organisms. It is described in detail how this approach may potentially be used to prevent or treat HIV infection, tuberculosis and cancer. The book is very well written in a clear, simple and stimulating style, which makes it accessible not only to immunologists but also non-specialists, teachers, medical students and clinicians. It will convince many that the immune system is not too complex to be understood. By Peter Bretscher. FriesenPress, 2016. 200 pp. ISBN: 978-1-4602-9656-1.

Peter Bretscher is a renowned immunologist who has been doing both conceptual and experimental immunology for almost fifty years. He is famous for the two-signal model of lymphocyte activation, which he published in 1970 together with Melvin Cohn. This model provided an explanation for how self/non-self-discrimination is realized by the immune system. Bretscher is an outstanding communicator whose ambition in his new book is to explain both basic immunological concepts and their relevance to medicine to a broad public. It is in many ways a shorter, simpler version of his previous and very successful book *Rediscovering the Immune System as an Integrated Organ,* which was published at the beginning of 2016.
……The book ends with a very stimulating 33 pages-long chapter dedicated to novel strategies for medical interventions. Bretscher makes a number of concrete suggestions for how conceptual immunology may be used clinically in domains as different as infectious diseases, allergy, autoimmunity, transplantation and cancer. The potential of immunological imprinting is first described. The Concept is based upon mouse experiments and human observations suggesting the immune response to a given antigen can be locked into a Th1 or Th2 mode……...
…… …It is described in detail how this approach may potentially be used to prevent or treat HIV infection, tuberculosis and cancer.

Section III: A Personal Perspective on the History of Two Signal Models of Lymphocyte Activation

This influential model, first published in Science in 1970, evolved, as put forward by Cohn and myself and others, as the years passed. Cohn also gave accounts of the history of how this model originally arose. I found these accounts in some respects to be misleading. I also felt that most of the modifications of the model, proposed by Cohn after I left his lab, to be implausible, and so undermining the appeal of the original model. I therefore decided, in the early 2010s, to write an article reassessing and reaffirming the 1970 formulation of the two signal model, in the context of then contemporary evidence and ideas. Cohn responded to this article, explaining why he disagreed with several of my proposals, and also making again what I considered inaccurate statements on its history. As a result of this, I decided to post my personal perspective of the history of the 1970 model in 2014 on my web profile. This was 44 years after the model was first published, and decades after Cohn's first accounts of its history that I felt to be inaccurate. This personal perspective constitutes the main body of the third section of the profile. I have not brought this section up to date, as I wanted it to stand as originally written. However, I think it appropriate to add some new facts here. Cohn died in October, 2018, at the age of 96. Robert Coffman, Stephen Hedrick and Alan Sher wrote a two page In Memoriam of Cohn, for the March/April Newsletter of the American Association of Immunologists. They described there how I came to Cohn's lab in 1968, as a postdoctoral fellow, with the basic ideas of the two signal model already formulated. This accords with my memory.

August 2019

The account below was written from November 2014 to April 2015

There are two related reasons for wishing to have an accurate history of how significant advances in a scientific field are made. The most obvious and prosaic is to give credit where credit is due. The second reason is more important and interesting. A knowledge of how important advances comes about is germane to understanding how progress occurs. Such understanding can be inspirational.

I think it useful to clarify at the outset what different people mean by "two signal models" in the context of lymphocyte activation, as my reading of the literature leads me to recognize there are two different and sometimes potentially confusing uses. Dresser and Mitchison, in their 1968 article in Advances in Immunology[1], clearly state the idea that competent lymphocytes can interact with antigen in two ways, one way leading to their "paralysis", or inactivation, and the other leading to lymphocyte activation. In this case, there are two separate kinds of signal a lymphocyte can receive, each with different consequences. I shall refer to this use as "a model for the two signals involved in the paralysis and activation of lymphocytes". Sometimes people refer to this idea

simply as a two-signal model, but it is clearly not a “two signal model of lymphocyte activation.” The term “two signal model” as now used is most often taken to mean a two-signal model of lymphocyte activation. It refers to the view that the activation of a lymphocyte requires antigen to interact with the receptor of a lymphocyte, generating signal 1, that, if generated alone for a sustained time, leads to the inactivation of the lymphocyte; the activation of the lymphocyte requires the generation of this signal 1, and a second signal, in addition. This second signal is referred to as signal 2. A major impetus for the genesis of this two signal model of lymphocyte activation was to provide an understanding, in 1969/1970, of how self-nonself discrimination might be achieved[2], as outlined below. This was a time when it was not recognized, as also outlined below, that there are central and peripheral mechanisms of tolerance.

The account I give here was stimulated in part by other accounts of how the two signal model of lymphocyte activation came about. It seems to me that these accounts are inaccurate, and therefore mitigate against a correct appropriation of credit. More importantly, these accounts obscure the considerations underlying the initial proposals, and so undermine an understanding of the imaginative, rational and observational considerations involved. A real appreciation of these considerations might stimulate other individuals, of a susceptible temperament, to be empowered to be brave, a quality required to be creative. This concern for accuracy may seem strange to some. For me this concern is very important, as my intellectual braveness, such as it is, was fostered by appreciating the braveness of others by following their intellectual journeys. This fostering led me to believe in the subtlety of argument. I felt I was a part of a community and culture that recognized the value of subtle thoughts. I hope a correct account of the origin of the two-signal model can help others to be brave, and thus beneficially contribute to this culture. I would like to add, in parentheses and so not to be misunderstood, that the best kind of braveness is probably unselfconscious; it arises from intense engagement with a problem and an unconscious faith, nurtured through experience, that subtle considerations can yield enormous dividends.

Cohn wrote an invited first article of reminiscences for the 1994 Annual Review of Immunology, entitled the Wisdom of Hindsight[3]. I read that part describing the origin of our Two Signal Model of lymphocyte activation shortly after Cohn’s article was published. Cohn’s account was much different from my recollections. I decided not to read the rest of the article. I had often found, after leaving the Salk Institute in 1973, Cohn’s papers difficult to read. I found them both difficult to understand and, when I thought I understood what Cohn meant, I often found the proposals put forward to be non-compelling or implausible. I therefore did not make the effort to give them great attention. I have thus not been much engaged in getting to know and critically assessing Cohn’s ideas after I left the Salk Institute in 1973. This might be surprising to some. There was also a more emotional reason. I found it difficult to read Cohn’s papers when I felt his proposals often muddied what we had proposed together in our 1970 Science article[2]. I also felt, for most of my career, that the best response, to my perception that our vision was being distorted in an unfortunate way, was to do the best science I could,

and not become distracted by taking on a personal agenda to put matters right. I found support for this course with my happiness at how we had expressed our vision in the Science article[3], our most cited, joint publication. When I have read it decades after it was published, I have no misgivings.

I had a year of administrative leave, starting in June 2013, from my normal duties at the University of Saskatchewan. In my normal university life I had been busy teaching, doing research and some administration. However, this administrative leave encouraged me to reconsider my priorities, particularly in view of my age of 70, and my frustration, over decades, that ideas, that I considered may be valuable to the field, were barely considered by the immunological community, particularly those ideas on immune class regulation. I also envisaged these ideas could have a considerable impact on medicine and the relief of human suffering. I therefore decided to devote considerable energy in this year to explore ways to get my views on two subjects more broadly considered. In the end, I found a way to get two reviews, describing my current thinking, accepted by the Scandinavian Journal of Immunology, as "Discussion Forums", one on the activation/inactivation of CD4 T cells[4], the other on Immune Class Regulation[5]. I did this on receiving the same suggestion from two individuals I substantially met for the first time during this year, Colin Anderson, of the University of Alberta, Canada, and Alexandre Corthay, of the University of Oslo, Norway. Both suggested I submit these articles through the Associate Editor, Zlatko Dembic, also of the University of Oslo. There is clearly a tradition, through the forums of this journal, to open up discussion of fundamental questions.

My first Discussion Forum paper, on the Activation/ Inactivation of CD4 T cells[4], drew a long response from Cohn[6]. I was asked to review it, as he had been asked to, and did, review my corresponding submission. I have therefore, in the last more than twelve months, become more familiar with both the details of some of Cohn's scientific proposals and also with his description of how the 1970 Two Signal Model of lymphocyte activation[3,6] came about. In view of the statements on the history of the two signal model for lymphocyte activation that Cohn made in his 2014 response[6] to my Discussion Forum article[4], and in his 1994 article in Annual Reviews of Immunology[3], I decided to embark on this project, to outline those statements of Cohn, on the history of the two signal model of lymphocyte activation, with which I disagree, and contrast them with my recollections of the pertinent events, with supporting evidence. I have not read other accounts of the history that Cohn has written since 1970. I consider that the two accounts I have before me is a sufficient basis for me to comment on.

Cohn, in his "The Wisdom of Hindsight" article[3], has a section entitled "The Origin of a 'Two Signal Model' of the Self-Nonself Discrimination". This section starts off with:

"Peter Bretscher joined my laboratory in 1967; he was, by way of background, an X-ray crystallographer who had a passionate curiosity about immunology as well as a clear,

crisp way of analyzing complex problems. This made the difficult question of the self-nonself discrimination an ideal one to answer; we were aware that it had to be analyzed correctly if a next step was to be made in immunology.

"We had two prior formulations, those of Lederberg and Forsdyke……..Forsdyke gave us the barest hints as to what a competing theory would entail…... Forsdyke proposed a situation where an interaction leading to a doublet [antigen binding to two distinguishable antibody sites of identical specificity in close proximity] results in inactivation, whereas one leading to a singlet leads to activation, in any given cell. ….A two signal mechanism distinguishing self from nonself by each antigen-responsive cell was required, and Bretscher and I set out to develop just such a theory." I take Cohn to mean here a model for the two signals involved in the paralysis and activation of lymphocytes, not a two signal model of lymphocyte activation.

I would now like to give an account of my recollections concerning the origin of the two signal model of lymphocyte activation. I apologize for going into considerable detail; such an exposition seems important for others to both understand the basis, and assess the legitimacy, of what I say.

My involvement

I became interested in immunology when a graduate student, under the supervision of John Kendrew, doing research in the field of protein X-ray crystallography, at the Cambridge Laboratory of Molecular Biology. I started my graduate studies in 1964. From the very beginning, I was much more interested in what was going on in the Division of Molecular Genetics than in the analysis of protein structure by X-ray diffraction. The Division of Molecular Genetics was headed jointly by Brenner and Crick. A group of research students and postdoctoral fellows, typically about 5 to 7 individuals, primarily from this division, met semi-regularly on Saturday mornings for a few hours to chat, with Sydney Brenner and over Nescafe, about diverse scientific topics. I joined my brother Mark in attending these get-togethers when I first became a graduate student. Mark soon left for a postdoctoral fellowship with Paul Berg, at Stanford, and I continued attending these meetings.

It was in 1966 that Sydney talked about a paper written by himself and Cesar Milstein on a model for "The origin of antibody variation", that had just been accepted by Nature. I thought about this model over the next week and came to believe it implausible, and the next Saturday I tried to explain my reasons to Sydney. Sydney was marvelous. He considered my reasons and thought them interesting. He took my proposal that there were likely to be invariant amino acid residues in the variable region of immunoglobulin chains to Cesar, a possibility unanticipated on and in weak conflict with their model; Cesar in time confirmed the validity of this proposal. The prevalent existence of such

invariant residues made the Brenner/Milstein model less plausible. This experience spurned my interest in immunology.

In time, I got into the habit of very occasionally making use of the invitation, placed on a notice-board outside Crick's and Brenner's joint office, to discuss science with Crick. I probably knocked on Crick's door on at least ten occasions.

As I developed various ideas about immunological subjects and about my PhD research, I took the opportunity to discuss these with Francis. One of my earliest immunological ideas now appears obvious. However, at the time, Francis appeared to think it new and was enthused. This is perhaps not so surprising, as at the time some reasonable people were unsure about the plausibility of the Clonal Selection Theory. My ideas buttressed the plausibility of some of the critical features of the Clonal Selection Theory.

I thought that when antigen interacted with antibody receptors on an antibody precursor cell, there must be "an interaction sensing site" that "recognizes" this interaction via changes in the structure of the antibody. I considered the situation that would occur as the genes coding for immunoglobulin chains are generated, in evolutionary time or somatically. I considered that this "interaction sensing unit" must be complementary to an invariant part of the antibody molecule; if it were complementary to a variable part, there would have to be two "complementary" mutations, one in the antibody genes, the other in the gene encoding the interacting sensing unit, an unlikely event. Assume, as seemed plausible, that the "interaction sensing unit" was indeed complementary to an invariant part of the antibody molecule. In this case, if the cell had two chemically different antibody receptors, the cell could not distinguish which of the two interacted with antigen, and so could not, for example, *uniquely* only activate the transcription of genes coding for the antibody receptor that had interacted with antigen. Thus I was led to the proposition one cell "must" be committed to the synthesis of antibody of just one specificity. This consideration was obviously another way of making plausible some essential aspects of the Clonal Selection Theory. These considerations formed part of the two papers Cohn and I subsequently published in Nature in 1968 and in Science in 1970.

Crick encouraged me, once he understood what I was driving at. Francis could initially not understand what I was saying, given my nervous disposition. He recommended I put my ideas in written form on one side of a page. I did this and gave him my effort. The argument was dense, and as rigorous as I could make it. He read it and in time understood what my considerations were. I of course remember the details of this interaction vividly, as it was so important to me. Francis gently chastised me. I did not need to take his recommendation of one page so literally, and so make the argument so dense. Neither did I have to prove my proposal, just to make it plausible. My interactions with Francis determined to a considerable extent my scientific fate.

On getting interested in immunology, I asked Cesar Milstein for advice on an introduction to the subject. He suggested Burnet, and lent me his copy (presumably) of The Clonal Selection Theory of Acquired Immunity. I read some it, but I was quite naïve. I remember I found it in parts implausible, and so did not read it in toto. I clearly remember though that I took notice of one remark made. Burnet pointed out that an antigen, in order to be immunogenic, i.e. able to induce the formation of antibody, had to be a macromolecule. This caught my attention, as small molecules could interact with antibody. The inference was, perhaps, that the simple interaction of antigen with antigen receptors was insufficient to activate an antibody precursor cell. I must have gleaned from Burnet the importance of the attribute of self-nonself discrimination. I probably was also introduced to the idea that the immune system relies on the early presence of self antigens in development, and their continuous presence thereafter, in ablating the ability to launch anti-self immune responses. This idea seemed so obvious to me that I now cannot remember its source. I also searched and read other articles, particularly after I had an accident.

This accident must have also occurred sometime later but still in 1966, I think. I was using a chemical that reacted predominantly with the free amino group of lysine residues of proteins. This chemical was somewhat dangerous, as it readily permeated skin. I wore gloves in using it. The gloves leaked. The tip of my right index finger started throbbing one night as I fell asleep, on a day I had used the chemical. The next morning the tip was white and quite dead. In the long run, the tip was surgically removed and skin, obtained from my arm, was grafted onto the raw end. However, before this, during the next few days after the mishap, a sharp line developed between what I imagined was healthy me and the proteins of my finger tip, altered by reacting with this chemical and so becoming “foreign”. This personal experience stimulated my interest in how the immune system might manage to respond to foreign but not to self-antigens.

My limited reading, or a conversation with Cesar Milstein, I now do not know which, led me to believe that antigen could interact with antibody precursor cells in two ways, one leading to the antibody precursor cell’s inactivation, the other to its activation, i.e. multiplication and the differentiation of its progeny into antibody secreting cells. There is one circumstance I recall that is surely significant. I had already decided to try to become an immunologist if I managed to get my PhD. In talking about this with Francis Crick, he suggested I might do postdoctoral studies with Avrion Mitchison, at that time at Mill Hill, London. I therefore visited Av, as he was known to all, sometime in 1966 or perhaps early in 1967. Av explained his lab was very full but that, if I really wanted to come, he could probably squeeze me in. I remember very clearly from this visit a big picture Av had high up on a wall. It represented a conclusion drawn from some of his recent studies. Av had harvested spleen cells from mice primed with the hapten carrier conjugate, h-C, and could show that such primed cells, when exposed to h-C in vitro for some time, could mount a secondary response to the hapten when subsequently given to irradiated mice without more antigen. Av found the presence of a monovalent form of h, when given in sufficient amounts with h-C during the in vitro stimulation of the primed

cells, could block the production of anti-hapten antibody when the primed cells were given to irradiated mice. This “again” implied that the interaction of a small molecule, the hapten, with an antibody receptor on the memory antibody precursor cell, was insufficient to “activate” the antibody precursor cell; actually, the hapten could block the precursor cell’s activation by h-C. I recall that I was at this stage already very interested in what was required to activate antibody precursor cells, but I had not yet formulated any clear ideas that were appealing under sustained examination. Av was in the future (at the 1967 CSH symposium) to articulate his view that the generation of a secondary antibody response required antigen to mediate cell-collaboration between hapten-specific antibody precursor cells and carrier-specific cells. I believe that Av had not yet come to this conclusion on my visit, most likely, or was reticent in openly expressing his views. I remember asking him what more was required to activate the antibody precursor cell than its interaction with the hapten/antigen?

I had some ideas, most probably in1967, and certainly after my meeting with Av, about how antigen might interact differently with antibody precursor cells to result in their activation and in their inactivation. I realized that the possibility that single precursor cells are inactivated on interacting with antigen, but that their activation required the antigen-mediated interaction between specific lymphocytes, had three appealing characteristics. Firstly, it was the only proposal I knew of that attempted to account for how antigen interacted differently with the same antibody precursor cell to result in either its inactivation or its activation. Secondly, it “explained” why antigens had to be macromolecular to be immunogenic. Thirdly, it accounted for how self non-self discrimination could be achieved, as outlined elsewhere[2,4,7]. Briefly, anti-self lymphocytes were envisaged to be inactivated when they were first generated, one, or a few, at a time, due to the early presence of self antigens in ontogeny and their continuous presence thereafter. Lymphocytes only able to recognize foreign antigens would accumulate during ontogeny in the absence of the foreign antigen and, once the foreign antigen impacted upon the immune system, this antigen could mediate the lymphocyte cooperation needed to initiate an immune response against the foreign antigen. The ideas concerning the activation and inactivation of lymphocytes occurred to me before I had come across any observations on “carrier effects”. However, I searched the literature for observations bearing on this proposal for one lymphocyte/multiple lymphocytes for the antigen-dependent inactivation/activation of lymphocytes. I did not know what journals to look at, so I initially randomly leafed through various periodicals on the shelves, looking for immunological articles. I found a paper in the Journal of Experimental Medicine that seemed to strongly support the proposal I envisaged. This was a paper by Benacerraf’s group on guinea pig non-responders to poly-L-lysine (PLL). These animals could make a response to PLL when they were immunized with PLL coupled to an immunogenic carrier, namely bovine serum albumin (BSA). There was no interpretation of the observations in the paper by the authors along the lines I envisaged. This was the first paper, among several, that seemed to support the ideas I had come up with.

Two review articles were very important to the further development of my ideas as a graduate student in Cambridge. Firstly, the article in the 1968 Advances in Immunology by David Dresser and Av Mitchison gave a very broad overview of observations and ideas pertinent to generating antibody responses and generating unresponsive states. Particularly important to me were the references to articles and the description of studies by Bill Weigle from 1961 onwards, as I have described elsewhere [4]. Many observations of Weigle fit in with the proposal I was entertaining, and had been in the literature for several years. There were no suggestions I could find anywhere, along the lines I envisaged for how these observations might be interpreted, for example in the Dresser and Mitchison article. This appreciation of the explanatory power of the ideas certainly increased my perception of their plausibility and potential significance.

I also came across an article by Gell and Kelus on Anti-antibodies in Advances in Immunology, 1967 (Adv Immunol 6:461-78.). I was very interested to know under what circumstances the interaction of antibody with antigen resulted in conformational changes in the antibody molecule, as I envisaged such changes had the potential for signaling to the precursor cell that its antibody receptor had interacted with antigen. I found the description, of antibodies that could recognize sites on other antibody molecules that were complexed with antigen, but these sites were not present on unbound antibody, very intriguing from this perspective. These anti-antibodies provided a means of detecting changes in antibody conformation that occurs when antibodies formed complexes with antigen.

As my reading of the immunological literature advanced, I kept Francis informed of how my ideas progressed. He changed his advice on where I might do my postdoctoral studies. Francis was now annually visiting the Salk Institute as a Non-Resident Fellow and, given his appreciation of my great interest in theory, suggested I might do a postdoc with Mel Cohn at the Salk Institute. Naturally, I accepted Francis' advice.

Mel Cohn also naturally accepted me on Francis' recommendation. Cohn had a major experimental, NIH-sponsored, program for producing myeloma tumors in BALB/c mice. He already had a substantial collection. I was encouraged to apply for a Damon Runyon Postdoctoral Fellowship whilst a graduate student in Cambridge. I had to outline a plausible research proposal. I suggested that the cause of the myeloma cancer might be because the cells produced a myeloma protein that had an abnormal structure, reflecting a structure representative of antibody complexed with antigen. I envisaged that if such abnormal "myeloma antibodies" were generated in an antibody precursor cell they might give rise to precursor cells that behaved as continually activated, and so constitute a cancer of this cell. The prediction was that myeloma proteins, not complexed with antigen, would react with antibodies that normally only recognize antibody sites present on antigen/antibody complexes. Although this idea was not experimentally explored, as I came to feel it implausible once I had been in Mel's lab for several months, it was part of my successful application for a postdoctoral fellowship, and this idea was reflected in my first publication with Cohn[7].

Before leaving Cambridge for southern California for my postdoc, I submitted a letter to Nature on a non-immunological topic. This letter was received by Nature on July 8, 1968. I surmise I arrived in Cohn's lab in late July, 1968, at the very earliest.

My impression, on arriving at the Salk Institute, was that Cohn was mainly pre-occupied with the problem of the origin of the diversity of antibody genes. He was less aware of the literature on the requirements to generate antibody responses and generate unresponsive states than I was. I had not come across, as a graduate student, Lederberg's classic Science paper of 1959[8]. I became aware for the first time of the proceedings of the 1967 Cold Spring Harbor Meeting. The two major "new" discoveries for me, from reading the proceedings of this meeting, was that others, namely Mitchison, Rajewsky and Jerne, had come to the view that the generation of an antibody response most probably required the antigen-mediated interaction between lymphocytes. Secondly, Rajewsky's contribution described observations, novel to me, concerning his work with porcine lactic dehydrogenase (LDH) isozymes as antigens in rabbits. Rajewsky's observations were of particular interest to me, as they were interpreted as showing that non-immunogenic molecules were non-tolerogenic, a proposition inconsistent with the ideas I had developed in Cambridge. I thought this conclusion misleading, for reasons we outlined in our 1968 Nature[7] and 1970 Science[2]papers, and that I have also recently discussed[4]. I might add that, in some real sense, Rajewsky's observations and their interpretation were not radically new, as somewhat similar observations had been made earlier, as recorded in the Dresser and Mitchison article, and been similarly interpreted.[1] I have recently given a detailed account of these observations and the considerations they led to.[4]

I was very motivated, on arriving in Cohn's lab, to quietly and thoroughly review the literature to see how my ideas might stand up to current findings and other people's ideas, and to explore how they might be further developed before "going public". This was not to be.

Cohn told me shortly after my arrival that he had been invited to a small meeting of about 40 individuals, on Immunological Tolerance, to be shortly held at Brook Lodge, Michigan. Moreover, Mel was asked to summarize the meeting. The invited participants to this meeting included many of the leading investigators, including Av Mitchison, Klaus Rajewsky, Bill Weigle and Benacerraf. The meeting was held from September 18 to September 20, 1968, surely less than two months after I had arrived in Mel's lab. It was natural for Cohn, in his summary of the meeting, to try to account for the major findings, outlined during the meeting, in terms of the ideas we were developing.

Cohn, on returning to the Salk Institute, gave me the original transcript of the meeting. I still have this transcript. On reading this transcript at the time, two things seemed clear. (All this is from memory; I have not gone back to check). Cohn's description of some critical observations from the literature was challenged, and he was not that clear in his

response to these challenges. In particular, I believe, he was unclear about the nature of Weigle's observations that showed that challenges with an antigen that crossreacts with the tolerogen could break the unresponsive state. This was a most critical finding that supported our proposals. This concerned me as it meant our model was not as clearly enunciated as it should have been. The major contention was caused by our view that non-immunogenic molecules could cause unresponsiveness. Most thought there were carrier effects not only in generating an antibody response, but in generating unresponsive states, or in causing the inactivation of the antibody precursor cell, a proposition to which I shall return. Cohn refers to this analysis on our part in a way that seems historically incorrect and to reflect an unawareness of the paradoxes of the late 1960s. He says (top of page 35, Annual Reviews of Immunology[3] volume 12: "The second choice [of a requirement for recognition of a carrier for paralysis] was initially rejected on experimental grounds (i.e. recognition of the carrier is required for induction, not unresponsiveness)." This statement misses, as far as I am concerned, the critical point evident to me at the time. Those few who had come to grips with carrier effects, namely Mitchison and Rajewsky, agreed that there was lymphocyte cooperation in the induction of antibody, but argued that *there were also carrier effects in generating unresponsiveness.* It seems to me Cohn misses the essential clarification "our" proposals brought about in recounting these events. It required careful, subtle and quantitative analysis to realize why the observations reported by, for example, Rajewsky, did not mean that there were carrier effects in the inactivation of antibody responses, as I have carefully recently outlined.[4]

Both Cohn and I were uneasy about how our ideas had been presented at Brook Lodge. Moreover, the proceedings of the meeting were to be published as a book. We decided in the circumstances to write an account of our ideas, as then formulated, as a paper to be submitted to Nature. Nature received our submission on October 17, 1968, less than a month after the Brook Lodge Meeting. It was published in the November 2, 1968, issue.

I received a personal letter from Av Mitchison, shortly after our Nature paper was published. He said "I think you deeply cloud the issue by using the carrier effect to account for the immunity/tolerance decision. I don't at all see why one cannot have all sorts of lovely carrier effects and also something else, e.g. non-specific stimulation from macrophages." Klaus Rajewsky had come to the Salk Institute straight from the Brook Lodge meeting to talk science. As clear from our Nature and Science papers, we disagreed with a conclusion Klaus had come to in his studies in rabbits with porcine lactic dehydrogenases as antigens. His observations were a particularly clear example of those employed by immunologists to support the proposition that *non-immunogenic molecules were also non-tolerogenic*. We argued that such observations were likely mis-interpreted, as quantitative considerations[2,4,7] could be employed to suggest the observations were not against the ideas I had formulated as a graduate student and, moreover, other observations supported these ideas. Furthermore, it seemed most plausible that most self-antigens were *tolerogenic but not immunogenic.*

The description above gives my best recollection of the events leading up to the publication of our Nature paper.

Our paper was submitted to Nature on October 17, 1968. I had been in Cohn's laboratory for less than four months, perhaps only two, and did not arrive in 1967, as Cohn states[3]. Our Nature paper pretty well reflected the ideas I brought with me, except there were references to the ideas of Mitchison, Rajewsky and Jerne on cell cooperation in the generation of antibody responses, and to Rajewsky's LDH observations, referred to above. We (meaning I) thanked Crick for discussions related to this paper.

Cohn says, in the quote from his 1994 Annual Reviews article, that I came to his lab in 1967, but I came in late July, 1968, at the earliest. He talks about the influence of Donald Forsdyke's paper, published in the Lancet in early 1968, as though we were aware of it. This is a rarely quoted paper that I first came across when reading Cohn's 1994 Annual Review article. I have to say I was really surprised when I first read what Cohn stated. I should say that Forsdyke's paper does not refer to any of the observations on carrier effects, observed in generating antibody responses, observations which supported the ideas I had formulated as a graduate student and which we discussed at great length in our Nature and Science articles. Neither do Cohn's accounts of how the two signal model of lymphocyte activation came to be formulated prominently refer to these observations on carrier effects. Cohn's account is at odds with the fact that I came to his lab with the idea that one lymphocyte/multiple lymphocytes are required for the antigen-dependent inactivation and activation of lymphocytes, and with the considerations that this proposal was supported by a number of observations in the literature.

I have never met Donald Forsdyke. However, circumstances arose where Donald Forsdyke and I communicated by email early in the 2000s. When circumstances led to our email interaction, I decided I had to let him know my perception of the impact of his 1968 Lancet paper on the origin of our ideas, as Cohn inferred[3] we were inspired by his ideas, but we had not quoted Forsdyke's paper in either our 1968 Nature[6] or 1970 Science paper[2]. The account Cohn gave of Forsdyke's influence has been taken up by some philosophers/historians of science[10,11], which is most unfortunate, as it obscures the grounds for the original proposal. Moreover, if influenced by Forsdyke's proposal, we surely should have and would have acknowledged his paper. Thus not only is Cohn's account incorrect, but his account makes it appear that Cohn and I were amiss in not giving credit where credit was due. I wrote the following email to Donald Forsythe in 2002.

02:27 PM 2002-11-14 -0600, you wrote:

Dear Donald,

Thank for your response. I am pretty interested in how ideas develop as you seem to be. The Coombs response [to Donald Forsdyke's ideas] is not so surprising. Why are scientists not enamored of proposals for clear answers to basic questions, independently of whether they are "correct"? My youthful dream was that discussing science would be like playing really well in a quartet, where the music is divine, and one's own contribution so fused as to be unknown!

I hope it is alright to be straightforward. I had the idea of the one hit/two hit whilst a graduate student in Cambridge, and talked to Crick about it. Mel went to the Brook Lodge symposium about two weeks (?) after I got there. He did not seem at that time to be so involved in the activation/inactivation questions, but he summarized the model in his summation of the meeting. This report of the summation went through much development after the meeting. (I must have the original transcript somewhere). In fact, Mel Cohn did not know many of the experiments on which I had based the model. I thanked Crick for discussions in this first paper. I was quite surprised to read Mel's account of how the two signal model developed in Ann Rev Immunol., because it does not fit in with my memory of how things occurred in major ways. I first learned of your Lancet paper in 1994. This is of course not to deny its insight, just to say I am very surprised about Mel's account.

I will make an effort to visit you if I get close to Kingston. I think we might have a really good time. Peter

The 1968 Nature paper was written fast and under the considerable pressure I have described. I was very soon consciously uneasy with its content. I consider its strengths to be its articulation that antibody precursor cells would be inactivated upon interacting with antigen alone, and that their activation required a second site be recognized by antigen-specific "carrier antibody". However, its weakness was the envisaged mechanism by which the activation of the antibody precursor cell was "made to be dependent" on the recognition of a second site on the antigen by the carrier antibody. We suggested that the carrier antibody held the antigen in a way that, when the antibody receptor of the antibody precursor cell bound to two antigen molecules, the receptor took on a "distorted" conformation, recognized by an "interaction recognition unit" of the cell. This proposal reflected ideas in my postdoctoral research proposal. The weakness of this model was that it predicted that self-antigens, with repeating epitopes that could distort the antibody receptors on interacting with them, would induce autoantibodies.

Cohn and I very soon realized, as soon as we relaxed (!), the indicated weakness of the model described in our Nature paper. We realized in time that there was really only one secure way of ensuring a requirement that the activation of an antibody precursor cell

needed the recognition of a second site on the antigen by carrier antibody. Such a recognition would be required if the activation of the antibody precursor cell was obligatorily dependent on events *following* the recognition of a second site on the antigen by carrier antibody. We envisaged these events could involve the delivery of short-range soluble molecules to the antibody precursor cell, and/or membrane/ membrane interactions between the antibody precursor cell and a cell to which the carrier antibody was attached. These suggestions were highly novel and appeared at first to be "extravagant"; however, they were supported by the subsequent discovery of interleukins and costimulaory systems.

Jacques Monod was a non-resident Fellow of the Salk Institute. Jacques was very interested in immunology and he and Mel knew each other very well. Mel had done a postdoc with Jacques, working on the lac operon. I very much enjoyed Jacques' scientific company, and I think he enjoyed mine. As a non-resident Fellow, Jacques visited the Salk Institute for a few weeks every year. In our discussions in 1969, Jacques suggested to us the importance, in getting our message over, that we develop a way of expressing the essence of our views independently of the particular details of how they might be realized. We recognized the wisdom of this suggestion. We therefore started to say that the interaction of antigen with the antigen-specific receptor of the antibody precursor cell resulted in the generation of signal 1, and the recognition of a second site on the antigen by carrier antibody resulted in the generation of signal 2. We of course envisaged that the sustained generation of signal 1 alone resulted in inactivation of the antibody precursor cell. Its activation required the generation of signals 1 and signal 2, constituting a two signal model/ mechanism of precursor cell activation. Signal 2 would be mediated by what came to be recognized as interleukins and costimulatory systems. I might add here that Forsdyke suggests[10] his model was one of the first two signal models. It was one of the first two signal models, one signal leading to lymphocyte activation and the other signal leading to lymphocyte inactivation, but it was not a model that proposed two distinct signals were required for the activation of lymphocytes.

By 1970, when we wrote our Science article, it was clear the antibody precursor cell was a B cell and that the helper cell a T cell. The Science article was written with the care one likes to bestow upon what one hopes is a significant paper. We thanked Monod for important discussions, and extended our considerations to both B and T cells. This article constitutes a careful justification of the original two signal mechanism/model of lymphocyte activation, written without pressure.

Cohn states[6] in 2014: "When, in 1968, I presented the "Two Signal" model, as Goran Moller had dubbed it, to a meeting that was meticulously recorded and published, all bedlam broke out." I am again surprised. The "Two Signal" model (of lymphocyte activation) was not formulated in 1968. Mel used a two signal formulation in his first presentation at Brook Lodge, in the sense of two different signals, one for the paralysis and one for the activation of lymphocytes, but this formulation was part of current

concepts, as evident from the Dresser/Mitchison review[1]. This two signal model does not imply the requirement of two distinct signals for lymphocyte activation. The formulation of the two signal model of lymphocyte activation was a subsequent, important and heuristic development that did not, to my knowledge, involve Goran Moller.

I make one final point for clarity. It became apparent from various observations, made after 1970, that there were at least two different processes contributing to self-tolerance. There was central tolerance, occurring in primary lymphoid organs, by self antigens present at sufficient levels in these organs to cause efficient clonal ablation of their corresponding lymphocytes. Other self antigens are more prevalent in the periphery. Lack of reactivity against such “peripheral” self antigens is achieved by mechanisms of “peripheral” tolerance. The two signal model of lymphocyte activation attempts to address how peripheral tolerance is achieved.

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Section IV: Selected Publications

This section contains a list and description of some of my publications, with the aim of providing a forum for discussion of some current, important issues in immunology (email: peter.bretscher@usask.ca). I include narrative comments to indicate the historical context of the observations made or of the ideas discussed, as well as comments directed at indicating features pertinent to current debate. My aim is to provide a description that is understandable by the interested, non-specialist.

Papers are listed under different headings, indicating their primary area of relevance

Area A: Lymphocyte activation/inactivation and immunological self-nonself discrimination

A1. Bretscher, P.A. and M. Cohn. 1968. Minimal Model for the Mechanism of Antibody Induction and Paralysis by Antigen. Nature. 220:444-8.

Synopsis: It was proposed that lymphocyte activation by an antigen A requires the antigen-mediated cooperation between the resting lymphocyte to be activated and other A-specific lymphocytes, and that the interaction of the resting lymphocyte with A alone results in its inactivation (paralysis in then current terms). It was argued that these proposals incorporate a mechanism by which the immune system can respond to foreign antigens and normally remain unresponsive or "tolerant" towards self-antigens, see A2 below under *Explanation of Self-Nonself Discrimination*.

A2. Bretscher, P.A. and M. Cohn. 1970. A Theory of Self-Nonself Discrimination. Science. 169:1042-49.

Synopsis: Develops the grounds for the two signal model of lymphocyte activation. This model represents a more correct formulation, according to my mind, of the potentially valid ideas of the 1968 paper (A1 above). It is proposed that an interaction of antigen with a resting lymphocyte, through its antigen-specific receptors, leads to the generation of signal 1, which, when generated alone, leads in time to the inactivation of the lymphocyte, rendering it refractory to activation.

Activation was envisaged to involve the obligatory generation of this signal 1, and a second signal, signal 2, delivered directly or indirectly following the recognition of a second site on the antigen by the cooperating or "helper" lymphocyte, see the Figure 1. (Note that terminology was then different from now. It was envisaged that thymus derived cells helped via the production of a particular class of antibody called "carrier" antibody.) Thymus derived T cells were identified as the cells that cooperate with B cells and allow the B cells to divide and produce humoral antibody. It was explicitly predicted that the antigen-dependent activation of helper T cells (e.g. resting CD4 T cells in

current terms) also requires similar antigen-mediated cooperation between these specific T cells and other specific “helper” T cells (see A5 and A6 below for tests of this prediction).

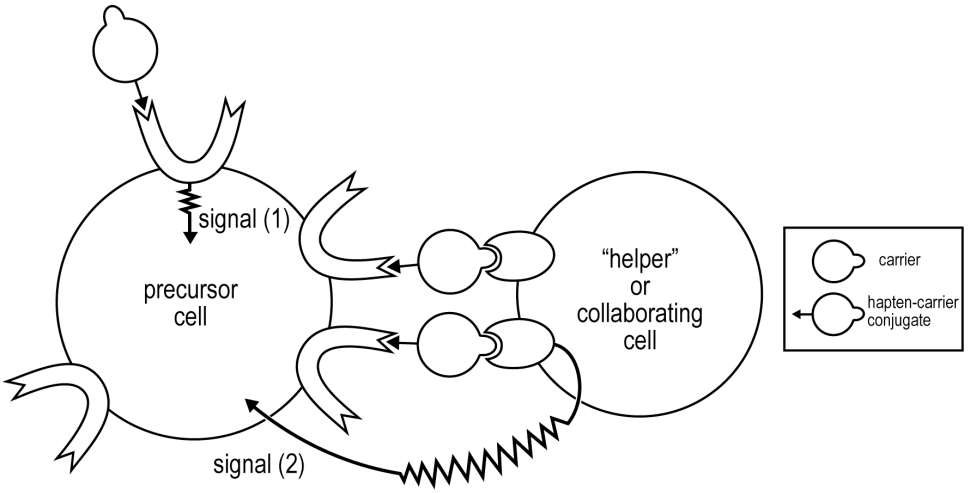


Figure 1 The original Two Signal Model of lymphocyte activation

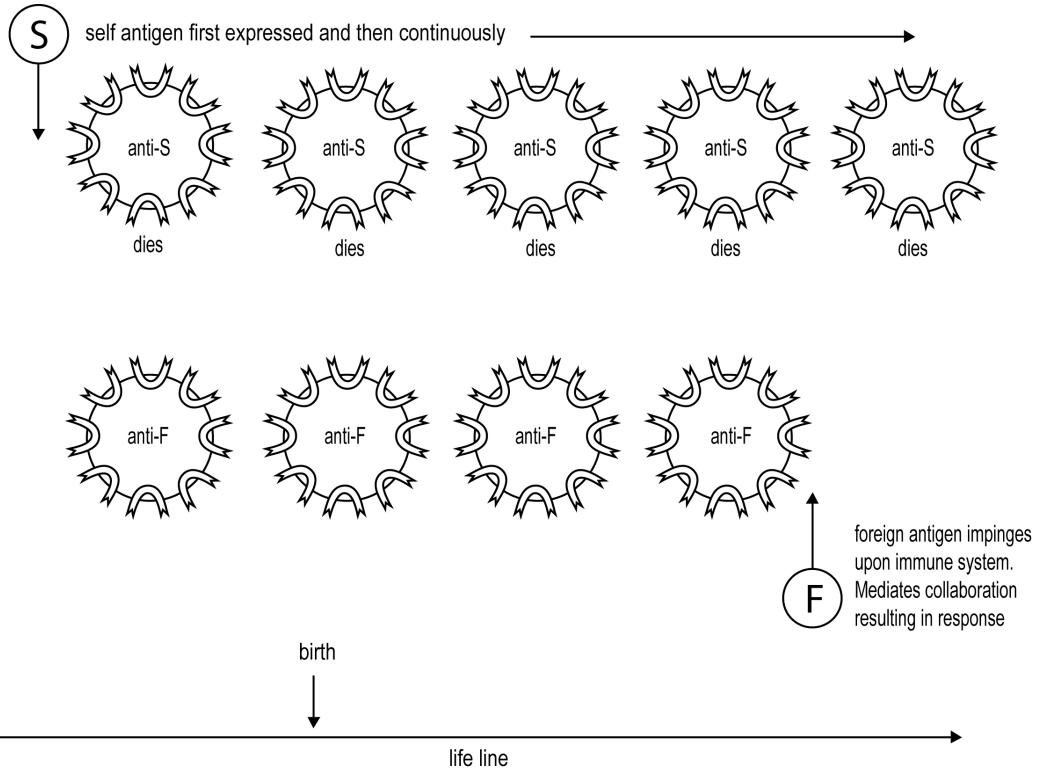


Figure 2 *Explanation of self-nonself discrimination according to the two signal lymphocyte model of lymphocyte activation. For explanation, see text.*

Explanation of self-nonself discrimination

The originators of the Clonal Selection Theory (Burnet, Jerne, Lederberg and Talmage) were preoccupied not only with how the genes encoding antibody chains were generated, but how the induction of anti-self reactivity could be prevented, see, for example, the classic and incomparably insightful paper by J. Lederberg, entitled Genes and Antibodies, Science, 129:1649 (1959). These individuals envisaged that the ability of the immune system to tolerate self- and respond to foreign antigens must rely on the fact that (most) self-antigens, in contrast to foreign antigens, appear early in development and are continuously present thereafter. This central idea is neglected or rejected by much contemporary analysis (as discussed under *The Historical Postulate* in A9 below). The two signal model accounted for peripheral self-tolerance thus, see Figure 2 above: lymphocytes specific for a continuously present self-antigen, S, are inactivated as they are generated, one or a few at a time. Lymphocytes specific for a foreign antigen, F, will accumulate in the absence of F. Once F impinges upon the immune system, F can mediate the interaction between the accumulated anti-F lymphocytes that is required to generate an anti-F immune response.

Current context: It was not appreciated, at the time this 1970 paper was published, that there are (at least) two levels of tolerance, namely central and peripheral tolerance. We now know that self-antigens, present in the primary lymphoid organs where lymphocytes are generated, i.e. the bone marrow for B cells and the thymus for T cells, can interact with their specific lymphocytes and inactivate them. This is called central tolerance. We also know that T cells are generated in the thymus specific for "peripheral", organ specific antigens, not present in the thymus at levels to cause reliable the reliable inactivation of these lymphocytes. These T cells emigrate to the periphery and secondary lymphoid organs, where they are kept in check by mechanisms collectively referred to as "peripheral tolerance". For example, a break-down in peripheral tolerance can lead to an immune response against the insulin-producing β-islet cells of the pancreas, resulting in diabetes. It seemed that, once the existence of both central and of peripheral tolerance was recognized, that the two signal model provided an explanation of peripheral tolerance.

A3. Bretscher, P.A. 1972. The Control of Humoral and Associative Antibody Synthesis. In G. Moller (ed.), Lymphocyte Activation by Mitogens, Transplantation Reviews, 11:217-267.

Synopsis: A general analysis of possible models of lymphocyte activation/inactivation in the context of achieving peripheral self-nonself discrimination is outlined. This conceptual analysis, together with references to pertinent observations, leads to the two signal model of lymphocyte activation. The antigen-recognition molecule made by T helper cells is here referred to not as the T cell receptor (TcR), as in current usage, or as "carrier antibody" (as in A2), but as "associative antibody". The paper also includes a

simple quantitative formulation of the two signal model. This formulation considers how the generation of signal 1 and signal 2 might depend on a variety of variables, such as antigen dose and the number/density of specific helper T cells. This quantitative formulation was central to the development of A Theory of Immune Class Regulation, see B1 below.

A4. Bretscher, P.A. 1975. The Two Signal Model for B Cell Induction. In G. Moller (ed.), Concepts of B Lymphocyte Activation, Transplant. Reviews, 23:37-48. This volume represents the then current views of different investigators as to the requirements for B cell activation. I attempt to critically examine the quality of the evidence, then available, for the two signal model, as it applies to B cells.

A5. Tucker, M.J. and P.A. Bretscher. 1982. The T Cells Cooperating in the Induction of Delayed-type Hypersensitivity act via the Linked Recognition of Antigenic Determinants. Journal of Experimental Medicine. 155:1037-49.

Synopsis: Tests of a critical prediction of the two signal model of lymphocyte activation as it pertains to T cells. This paper shows that the generation of (CD4+) T cells, able to mediate DTH and specific for an antigen A, could be helped by (CD4+) T cells specific for a non-crossreacting antigen B in the presence of the conjugate A-B, but not when A and B are present but unconjugated. This form of recognition between interacting lymphocytes is referred to as the *recognition of linked epitopes*.

A6. Bretscher, P.A. 1986. The Primary Induction of Helper T Cells Involves the Linked Recognition of Antigenic Determinants. J of Immunology. 137:2726-2733.

Synopsis: This paper demonstrates the existence of a cascade of T cell/T cell interactions in the generation of helper T cells. Each step in the cascade involves the antigen-mediated interaction of T cells with other T cells. Each of these interactions occurs via the recognition of linked epitopes by the interacting T cells. See synopsis of A5 above for an operational definition of recognition of linked epitopes, and A8 below for a possible mechanism by which such an operational recognition could be realized.

A7. Bretscher. P.A. 1992. The Two-signal Model of Lymphocyte Activation Twenty-one Years Later. Immunology Today 13:74-76.

Synopsis: Much evidence supports the two signal model, in that CD4 T helper cells are required to facilitate the antigen-dependent activation of most B cells and at least some CD8 T cells. In the absence of such CD4 T helper cells, antigen can inactivate the B cells and CD8 T cells, via the generation of signal 1 alone. These findings are consistent with the envisaged role of CD4 T helper cells as the guardian over the activation/ inactivation of other lymphocytes. The requirements for the activation/inactivation of CD4 T helper cells thus seems ever more central.

Evidence is reviewed showing that the activation of CD4 T cells most probably requires two signals. Signal 1 is generated when the T cell receptor (TcR) binds a peptide/MHC class II molecule complex on the antigen presenting cell (APC). The CD4 T cell is operationally inactivated when this signal is generated alone. Evidence supports the suggestion that CD4 T cell activation requires the T cell to receive a second signal, generated when a receptor on its surface binds to a “costimulatory molecule” on the APC. Both the costimulatory molecule and the T cell receptor recognizing it are envisaged in one model to be constitutively present on mature APC and on CD4 T cells respectively. According to some other models, the costimulatory molecule on the APC is inducible under some circumstances (discussed under A8 and A9). The model in Figure 3, in which the costimulatory molecule is constitutively expressed, is therefore conveniently referred to as the Constitutive Model.

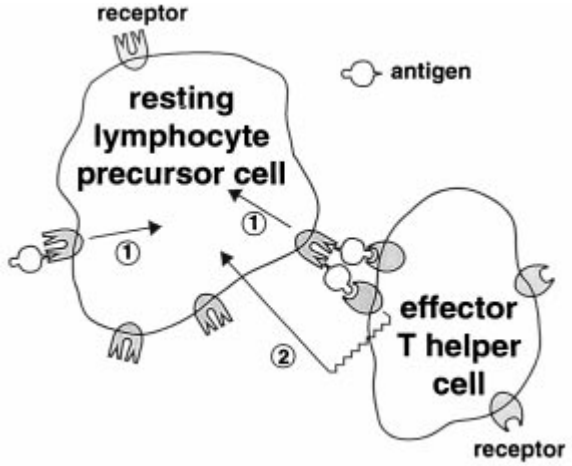


Figure 3 The Constitutive Model of CD4 T cell activation

I found this constitutive model problematical as a description of the activation/ inactivation of CD4 T cells. The description conforms very nearly to what was envisaged in the “original” Two Signal Model, see A2 above and Figure 2. However, although two signals are envisaged as being required for CD4 T cell activation in both the constitutive model and the “original” Two Signal Model of CD4 T cell activation, the nature of the second signal is critically different in these two models. In the original two signal model, signal 2 was generated and delivered following the recognition of a second site on the antigen by an antigen-specific lymphocyte. In the constitutive model, the second signal is delivered by the APC. The APC, being non-specific, will deliver signal 2 to CD4 T cells specific for self or foreign peptides alike. APC such as dendritic cells cannot distinguish between self and foreign peptides by themselves. It would appear such APC cannot deliver signal 2 in a manner that ensures the inactivation of CD4 T cells specific for peripheral self and the activation of CD4 T cells specific for foreign peptides. Also, this model ignores the evidence for T cell cooperation in the activation of T helper cells (e.g. see A5 & A6 above). This paper expresses the anguish of this predicament.

A8. P.A. Bretscher. 1999. A two step, two signal model for the primary activation of precursor helper T cells. Proc. Natl. Acad. Sci, 96:185-190.

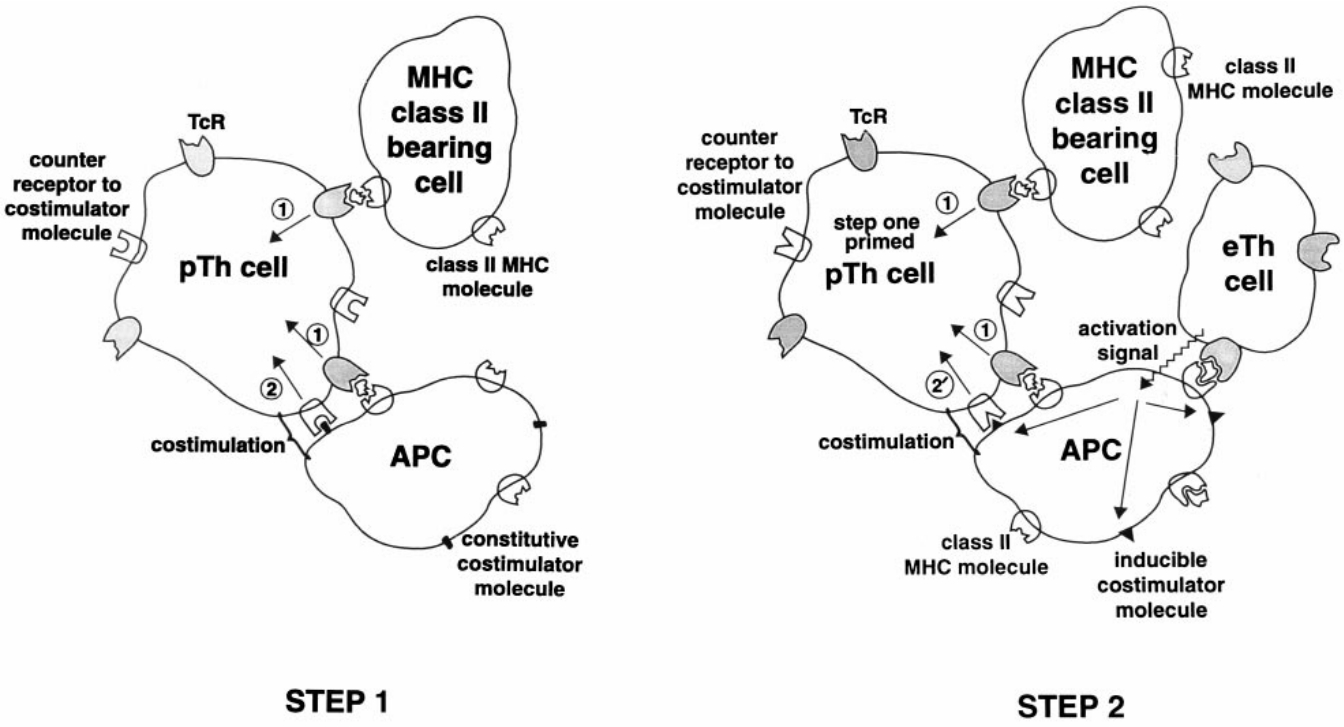


Figure 4 The Two Step, Two Signal Model for the activation of precursor T helper (pTh) cells

Context and synopsis: It was generally accepted, at the time this model was proposed, that the "original" two signal model of lymphocyte activation (see A2 above) applied to most B cells and at least some CD8 T cells (see A7 above). Thus CD4 T helper cells were required for antigen to activate most B cells and at least some CD8 T cells, and antigen could inactivate these B and CD8 T cells in the absence of "helper T cells". Thus CD4 T cells controlled, in large measure, the fate of other lymphocytes.

This two step, two signal model, see Figure 4, was an attempt to account for the pertinent, extant observations, and to resolve 5 problems.

(i) Addressing the problem associated with the constitutive model Observations support the idea that mature macrophages and/or dendritic cells constitutively express (B7) costimulatory molecules, and that antigen presented by these antigen presenting cells (APC) can induce immune responses. These observations led to the "constitutive model" (see A7). However, this constitutive model cannot account for peripheral CD4 T cell self-nonself discrimination (see A7 above). Suggested resolution: This "constitutive

model" describes the first step in the activation of naïve CD4 T cells, or precursor T helper cells (pTh) cells, thus accounting for most of the observations supporting the constitutive model. The first step is envisaged to result in the proliferation of the pTh cell, but these progeny are postulated to die or become anergic unless they successfully complete the second step. The second step is obligatory if these progeny are to give rise to effector Th (eTh) cells. The second step incorporates a mechanism of self-nonself discrimination, see (ii) below.

(ii) How to achieve peripheral self-nonself discrimination for CD4 T cells? The "original" two signal model (A2) proposed that (peripheral CD4) T cell tolerance could be achieved if inactivation occurred when antigen acts on a single CD4 T cell, and if the activation of a CD4 T cell required collaboration with other CD4 T cells. These "rules" incorporate a mechanism of self-nonself discrimination, as explained in A2 above. These "rules" are embodied in step two, see Figure 4, of the Two Step, Two Signal Model. Activation to yield functional effector Th (eTh) only occurs if the second step is completed, requiring the presence of other CD4 T cells (eTh) cells specific for the antigen.

(iii) The scarcity problem A problem recognized when the original two signal model was formulated arose from the fact that antigen-specific lymphocytes are expected to be very scarce in an "unprimed" animal, and yet the activation of pTh cells was envisaged to require the antigen-mediated interaction between such scarce Th cells. The first step results in an expansion of pTh cells, making these cells less scarce; this may favor their interaction with other scarce cells, thereby contributing to solving the scarcity problem.

(iv) The priming problem Biology contains many "priming" problems, the major one being the origin of life. We know life can beget life, but how did the first life arise? We need ribosomes to make ribosomes, but how did the first ribosome arise? We need, according to the Two Step, Two Signal Model, activated, i.e. effector Th (eTh) cells, to activate pTh cells to yield more eTh cells. How does the first eTh cell arise? We call this the priming problem. In this context, we first note that there is evidence for a requirement for eTh cells to initiate immune responses, as reviewed in this paper. Secondly, various ways of "solving" the priming problem are discussed in the full text referred to in A3 above.

(v) How can the recognition of linked epitopes between interacting CD4 T cells be achieved? Observations demonstrate that the antigen-mediated interaction between a pTh cell and the eTh, required to activate the pTh cell, occurs through a mechanism involving the recognition of linked epitopes (see A5 and A6). This was a critical prediction of the "original" two signal model. We first consider why linked recognition is so critical for understanding CD4 T cell peripheral tolerance. Then we discuss potential mechanisms of linked recognition.

Consider the situation where two pTh cells are newly generated, one specific for a peripheral self-antigen, S, and the other specific for a foreign antigen F, and where both F and S are present. According to the model proposed, there must be eTh cells specific for F in order for F to be able to activate the anti-F pTh cell. This would also result in the activation of the anti-S pTh unless the interaction between the anti-F pTh cell and the anti-F eTh cell required the recognition of linked epitopes. Recognition of linked epitopes is required to ensure that the process of activating pTh cells specific for foreign antigens does not inevitably ***interfere*** with the inactivation of pTh cells specific for peripheral self antigens. We refer to this desirable situation as the **Principle of Non-Interference**. Versions of two signal models that violate this principle seem to have the very same characteristic that makes third signal models (see A3) so unappealing: initiation of an immune to foreign antigens provides circumstances under which immune responses to peripheral self antigens would inevitably occur. Moreover, we appear to be able to rest in peace, as observation demonstrates a requirement for linked recognition!

To imagine a mechanism responsible for linked recognition has seemed problematical in the context of modern knowledge: CD4 T cells recognize a peptide, derived from the nominal antigen, bound to an MHC class II molecule. Consider a protein antigen Q that is processed into peptides q1,q2, q3,…..qn, recognized by CD4 T cells in the context of the host's class II MHC molecules. How can we imagine a mechanism by which an anti-q3 pTh cell can only be helped by an anti-q2 eTh cell, when Q must be broken down into q peptides before either Th cell can recognize its peptide? It seems the linkage, deemed so important, must be destroyed before the Th cells can interact.

I have been able to come up with only one plausible solution to resolve this issue. It consists of the proposition that the antigen presenting cell in the second step has to be an antigen-specific B cell. Consider the interaction between two CD4 T cells, one specific for the nominal antigen Q and the other for R, where Q and R do not crossreact. This CD4 T cell interaction would be mediated only by the Q-R conjugate. The antibody receptors of a B cell, specific for Q or for R, will be able bind the conjugate Q-R, and present peptides derived from processing Q and R. Thus a specific B cell could mediate the interaction between two CD4 T cells, one specific for Q and the other for R. Such an interaction would not take place in the absence of the Q-R conjugate and in the presence of separate Q and R molecules. The proposal that an antigen-specific B cell acts as the APC thus explains why the interaction between the CD4 T cells requires the recognition of linked antigenic epitopes. We suppose the requirement for a B cell can be assured by its expression, when activated, of a particular constellation of costimulatory molecules (and/or cytokines) required to activate the step one primed pTh cells through the second step. We have tested various predictions of this model, see A10.

A9. Bretscher, P.A., 2000. Current models for peripheral tolerance and the "classical historical postulate", Seminars in Immunology, M Cohn and R Langman, Editors, 12:221-229, 273-276, 325-329

This volume consists of initial statements by the invited participants, which were then made available to the other participants for their comments, and so on for a total of three rounds.

Synopsis The originators of Clonal Selection Theory envisaged that the ability of the immune system to tolerate self and respond to foreign antigens must rely on the fact that (most) self antigens, in contrast to foreign antigens, appear early in development and are continuously present thereafter. Consider, from this perspective, what must happen when two CD4 T cells, newly generated in the thymus, and one being specific for a peripheral self antigen, S, and the other for a foreign antigen F, emigrate to the periphery and encounter an environment containing both F and S. We would hope that S and F can inactivate and activate their respective pTh cells. Moreover, these different consequences should reflect the fact that S, in contrast to F, has been continuously present during the past *history* of the individual whose immune system we are considering. We refer to this idea, put forward by the originators of Clonal Selection Theory, as the "classical historical postulate".

The two step, two signal model of pTh activation provides an explanation of peripheral, CD4 T cell tolerance consistent with the classical historical postulate, see A2. Janeway proposes that the initiation of an immune response *requires* the activation of innate defense mechanisms through their recognition of pathogen-associated molecular patterns (PAMPs) of microbial pathogens (infectious entities), and Matzinger envisages that initiation requires the generation of a "danger signal". According to these "third signal" models, the most widely entertained models of the day, the third signal activates the APC to generate an effective costimulatory signal; antigen is envisaged to inactivate pTh cells in the absence of costimulation, i.e. in the absence of signal 3.

These third signal models postulate that ***only*** the circumstances at a particular time determine whether antigen activates or inactivates pTh cells at this time, i.e. the critical circumstance is whether a third signal is generated at this time, and the past history of the individual with respect to exposure to the antigen in question is not central or even relevant. These third signal models thus violate the classical historical postulate. I personally believe that activation of innate defense mechanisms, the occurrence of inflammation, or events arising from circumstances covered by the rather flexible concept of danger, can significantly affect immune responses. It is implausible to me, however, that third signals play the critical and universal role ascribed to them in third signal models, as these signals are not antigen-specific. I also discuss in this contribution observations difficult to reconcile with these third signal models.

A10 Testing predictions of the Two Step, Two Signal Model (A8)

A10a. **Power CA, Grand CL, Ismail N, Peters NC, Yurkowski DP, et al. (1999) A valid ELISPOT assay for enumeration of ex vivo, antigen-specific, IFN-gamma producing T cells. J Immunol Methods 227: 99–107.**

A10b **Peters NC, Hamilton DH, Bretscher PA (2005) Analysis of cytokine-producing Th cells from hen egg lysozyme-immunized mice reveals large numbers specific for ''cryptic'' peptides and different repertoires among different Th populations. Eur J Immunol 35: 56–65.**

A10c **Peters NC, Kroeger DR, Mickelwright S, Bretscher PA (2009) CD4 T cell cooperation is required for the in vivo activation of CD4 T cells. Int Immunol 21: 1213–1224.**

A10d **Kroeger DR, Rudulier CD, Peters NC, Bretscher PA (2012) Direct demonstration of CD4 T cell cooperation in the primary in vivo generation of CD4 effector T cells. Int Immunol. 2012/04/25 ed.**

A10e **David R. Kroeger, Christopher D. Rudulier, Peter A. Bretscher (2013) Antigen Presenting B Cells Facilitate CD4 T Cell Cooperation Resulting in Enhanced Generation of Effector and Memory CD4 T Cells, PLoS ONE 8: e77346**

A10f **Peter A Bretscher The Activation and Inactivation of Mature CD4 T cells: A Case for Peripheral Self–Nonself Discrimination Scand J Immunol 79: 348**

A10g **Ghassan A. Al-Yassin and Peter A. Bretscher (2018) Does T Cell Activation Require a Quorum of Lymphocytes? J Immunol 201: 2855**

A10h **Peter A Bretscher (2019) The history of the two-signal model of lymphocyte activation: A personal perspective Scan J Immunol 89:e12762**

Synopsis. We describe the above 8 papers together, as they are all pertinent to testing predictions and discussing the plausibility of the Two Step, Two Signal Model of CD4 T cell activation (A9)

We set out to test whether the *in vivo* activation of CD4 T cells requires CD4 T cell collaboration on immunization with antigen but ***without microbial adjuvant.*** Our previous studies on CD4 T cell cooperation had all been conducted in vitro. We judged we would need a very sensitive assay to detect activated CD4 T cells, and decided to employ the ELISPOT assay, developed by others to detect single, antigen-specific, cytokine-producing cells. We established the assay in the lab, but found that the number of "antigen-dependent spots detected", corresponding to antigen-specific cytokine-producing cells, dropped much more rapidly than the dilution of spleen cells plated. We

found that, if we supplemented the sensitized spleen cells with unprimed spleen cells, so the total number of cells plated was a constant, including the APC involved in the antigen-dependent stimulation of CD4 T cells, the sensitivity of the assay increased and the number of spots developed was now linear with the number of sensitized cells plated. This paper thus describes how the standard ELISPOT assay can be modified to validly enumerate antigen-specific cytokine producing cells **(A10a)**.

We wanted to define the peptide specificity of all the activated CD4 T cells generated, upon in vivo immunization with a protein antigen, and we chose hen egg lysozyme (HEL) as the antigen. We thought that such a definition might allow us to examine whether T cells specific for one peptide helped in the activation of T cells recognizing another peptide, on immunization with HEL. We employed the ELISPOT assay to define the peptide specificity, in both CBA and BALB/c mice, of the CD4 T cells generated upon immunizing with HEL. We validated our analysis by showing that the sum of the number of CD4 T cells generated to non-overlapping HEL peptides was equal to the number of CD4 T cells generated against the whole protein, in a spleen cell population from a mouse immunized to HEL **(A10b)**. Important for our further studies was the fact that, in BALB/c mice, half the CD4 T cells, generated upon immunization with HEL, were specific for the "major peptide", and the rest for "minor peptides" we identified.

HEL is a small protein, and not very immunogenic. We envisioned that CD4 T cells specific for HEL were sparse. We hypothesized that, if we ablated the CD4 T cells specific for the "major peptide" before immunizing with HEL, not only would the generation of the CD4 T cells specific for the major peptide be ablated, but the CD4 T cell response to minor peptides would be considerably diminished; we envisaged that the activation of CD4 T cells specific for these minor peptides were most probably facilitated, in a mouse with a normal complement of CD4 T cells specific the major peptide, by major peptide-specific CD4 T cells. We tested this prediction. We showed that when we ablated the CD4 T cells specific for the major peptide, the CD4 T cell responses to both the major and minor peptides were dramatically diminished following a challenge with HEL **(A10c)**.

Our next study demonstrated that CD4 T cells specific for a peptide of ovalbumin (OVA) could facilitate the activation of effector CD4 T cells specific for the major HEL peptide **(A10d).** We further examined the nature of the antigen-presenting cell (APC) mediating this cooperation. In order to approach the nature of the APC, we gave the peptide antigens in the form of APC loaded in vitro with these peptides. We successfully tested two main predictions. Firstly, a mixture of different APC types could only facilitate the cooperation between the CD4 T cells if the APC were loaded in vitro with both peptides; a mixture of two populations of APC, each loaded with one of the two peptides, did not facilitate the cooperation. This study tests the idea that the two CD4 T cells interact with each other via an APC that presents both peptides. We also tested

the ability of dendritic cells (DC) and B cells, loaded with the two peptides, to mediate the cooperation between the two CD4 T cell populations. We found that loaded B cells, but not loaded DC, could mediate the cooperation **(A10e)**. This study confirms a strong prediction of The Two Sep, Two Signal Model (A8).

The Two Signal Model of Lymphocyte Activation is widely accepted by the immunological community in the context of the activation of B cells and CD8 T cells, but not in the context of CD4 T cells. The most widely entertained, contemporary models for the activation of CD4 T cells are the Danger and PAMP (pathogen-associated molecular pattern) models. Moreover, since the publication of our two signal model in Science, in 1970 (A2), several variations of the original model have arisen. I felt and feel most of these variations undermine the coherence of the model as originally proposed. I wrote an article in 2014, 44 years after the model was first published, to re-examine and then support the essential ideas of the 1970 two signal model, both in the context of CD4 T cells, and of then contemporary information and ideas **(A10f)**. It also seems that contemporary studies are almost exclusively undertaken at the molecular and/or cellular level, ignoring past and current studies and ideas that are best described as at the level of the system. I shall shortly explain what I mean by “at the level of the system.” My PhD graduate student, Ghassan Al-Yassin, and I wrote a paper in support of the view that older studies, many at the level of the system, conflict with the Danger and PAMP models. I will illustrate our view with one example. William Weigle published several papers in the early 1960s, employing rabbits unresponsive to bovine serum albumin (BSA), following the administration of massive doses of this antigen on the first few days of life. The rabbits, as young adults, would not produce antibody on challenge with BSA, but they produced antibody to human serum albumin (HSA) on immunization with this antigen. Some of the antibody so raised to HSA bound to BSA. These are some of the classical experiments showing that immunization, with antigens cross-reacting with the antigen the animal is unresponsive to, can (sometimes) break the unresponsive state. There was, at the time, no cellular or molecular interpretation by Weigle, or anyone else, as to how this might occur. These studies were in this sense carried out at the level of the system. Such studies would not be considered important today by most immunologists, as they do not lead, as described, to a picture of what is happening in terms of cells and molecules. However, we argued that this and related studies really supported the proposition that the activation of helper T cells requires lymphocyte cooperation. We describe many older studies, many at the level of the system, that are difficult to reconcile with the Danger and PAMP Models, but support the idea the activation of T helper cells requires T cell collaboration **(A10g)**.

Area B: Immune class regulation

B1a. Bretscher, P.A. 1974. On the Control Between Cell-mediated, IgM and IgG Immunity. Cellular Immunology. 13:171-195

B1b. Bretscher, P.A. 1981. The Significance and Mechanisms of the Cellular Regulation of the Immune Response. Theoretical Immunology Symposium of the 64th Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, California, April 16, 1980, Federation Proceedings, 40:1473-8.

Synopsis These two papers provided a conceptual framework that attempts to describe how antigen interacts with lymphocytes to determine the class of immunity induced. At the time, the expression of delayed-type hypersensitivity (DTH) was used to indicate the generation of a cell-mediated response, and IgG antibody production to indicate humoral immunity. Much experimental work of my students and myself over decades has been directed at testing the theory I proposed here, and using it in attempts to achieve practical aims. I describe the theory in a manner to highlight predictions highly characteristic of it. I refer for convenience to the theory as a Theory of Immune Class Regulation. (It should be noted that the MHC-restricted recognition of antigen by T cells had not been discovered in 1974.) This theory is easily recast now, taking into account this feature of T cell recognition, by employing The Two Step, Two Signal Model, see B6 below, as a context for discussing ideas on immune class regulation. However, the original description and that given here is made within the context of the assumption that T cells recognize antigen in a similar way as antibody. It is convenient to consider three different types of consideration that were incorporated into the Theory of Immune Class Regulation.

(i) The mechanism of exclusion between cell-mediated and humoral immunity

It had been known for decades that different circumstances of immunization give rise to different kinds of immunity, i.e. either to predominant cell-mediated or humoral immunity, or to a mixed response. Then, in the mid 60s, a number of reports showed that the generation of humoral immunity precluded the subsequent generation of cell-mediated immunity in the form of DTH. Asherson and Stone christened this phenomenon with the name “immune deviation”. We now refer to this as “humoral immune deviation” to indicate the nature of the deviation. Chris Parish and colleagues described the related phenomenon of “cell-mediated immune deviation” in the early 70s. They repeatedly immunized rats with a protein antigen, in a manner that lead to cell-mediated immunity alone. Such sensitized rats, when given a challenge of the protein that generated a brisk antibody response in naïve rats, produced little antibody but the rats expressed DTH, i.e. expressed cell-mediated immunity to the protein. This represents a state of cell-mediated immune deviation. The phenomena of cell-mediated

and humoral immune deviation show that the immune response to a particular antigen can become locked into a particular mode, either humoral or cell-mediated. It was proposed in the Theory of Immune Class Regulation, partly on the basis of observation, partly on conceptual grounds, that the operation of distinct “suppressor” T cells were responsible for cell-mediated and humoral immune deviation. It was envisaged that chronic immunization, giving rise to a predominant cell mediated response, also gave rise to the generation of antigen-specific T cells that *suppress* or inhibit the corresponding humoral response. We referred to these inhibitory T cells as TsAb cells. It was also envisaged that the generation of a predominant antibody response gave rise to antigen-specific T cells that suppress or inhibit the corresponding DTH response. We refer to such cells as TsDTH cells see Figure 5.

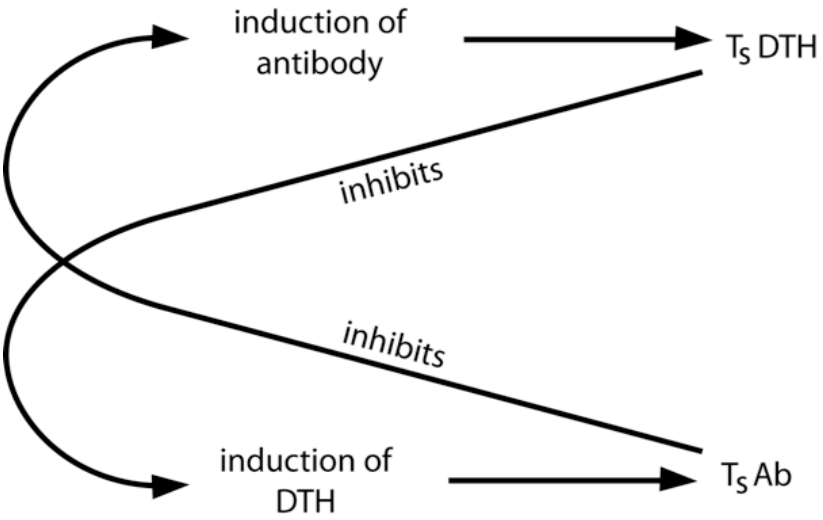


Figure 5 How TsAb and TsDTH are envisaged to account for the tendency for exclusivity between DTH and antibody responses

(ii) The Pathophysiological Significance of Immune Class Regulation It is perhaps initially surprising that the immune system incorporates decision making processes controlling which, of the diverse means it has of fighting foreign invaders, to employ. One might imagine that, once the “decision” has been made to fight a foreign invader, it would be advantageous to employ all means available. Observation shows this not to be so. Why?

A series of observations are suggestive of a possible answer. Antibody is ineffective against antigens that have few, or a low density (if a cell), of foreign sites recognized by the antibody. One example is the IgG antibody-dependent complement-mediated lysis of foreign red blood cells. A few IgG molecules, bound close together on the cell surface of the red cell, forming an IgG complex, are required to initiate the complement cascade. This initiation leads to the local generation of a hydrophobic “donut” that inserts itself into the red cell membrane, leading to the lysis of the red cell. Antibody can

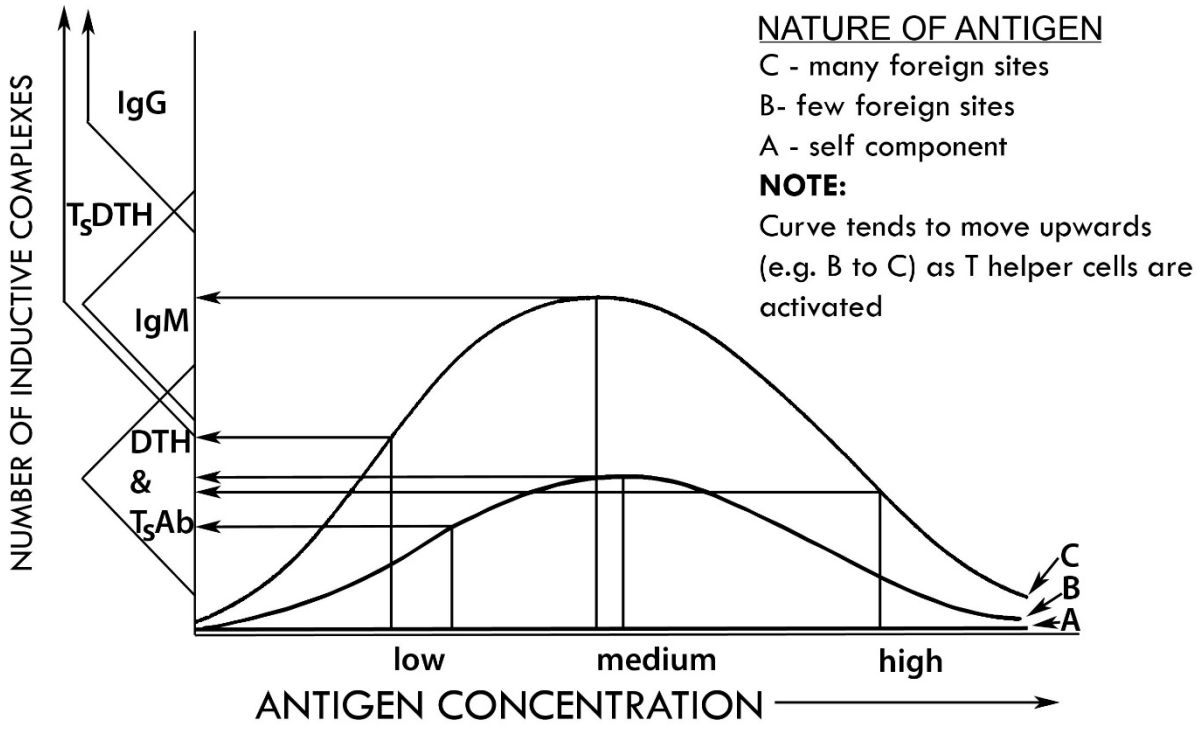
similarly attack bacteria. The requirement for an IgG complex to activate complement means that several 100,000 IgG molecules have to bind to one red cell in order for there to be a 50% chance of an effective IgG complex to form. Thus antibody is ineffective against cells with few foreign sites. Antibody is usually ineffective, for example, against cancer cells. However, cancer cells can be lysed by the cytotoxic T lymphocytes (CTL), an expression of cell-mediated immunity. I suggested that antibody is not normally induced to soluble antigens/cells with few foreign sites. This suggestion was made on the basis of observations by others, and because, in addition, it made sense in that it appeared antibody is likely to be ineffective in damaging such foreign invaders. Moreover, the presence of antibody might block the effectiveness of the cell-mediated response. This consideration provides a rationalization for why antibody production is often inhibited during the course of a cell-mediated response. However, antigens with many foreign sites can be attacked effectively by antibody-dependent mechanisms and can induce antibody. It is explained in this paper why the inhibition of cell-mediated immunity, during the course of a strong antibody response, can be rationalized as a means of minimizing the damaging effects of any autoreactivity induced.

Two potential insights follow from these considerations. Sometimes ineffective antibody is generated against tumors, or against pathogen-infected cells bearing pathogens that cause chronic disease (e.g. HIV-1 leading to AIDS*, Mycobacterium tuberculosis* leading to tuberculosis), resulting in stable or progressive disease. It was suggested that vaccination/ treatment might be achieved by ensuring a predominant cell mediated response. Approaches to achieve such ends are discussed in section C. It was also anticipated that humoral, antibody-mediated autoreactivity might *sometimes* be benign, whereas cell-mediated autoreactivity to the same antigens would more likely cause damage and hence autoimmune disease. This also seems to be sometimes the case.

(iii) The decision criterion controlling the cell-mediated/humoral nature of the immune response: the necessity for quantitative considerations

What determines whether antigen preferentially generates a cell-mediated (with associated TsAb cells) or an antibody, humoral response (with associated TsDTH cells)? Firstly, it was argued that TsAb and TsDTH cells probably inhibit each other's generation so that, once one class of cell becomes a bit dominating, it inhibits its adversary and so tends to become more dominant, see Figure 5. It was also argued that if antigens with few foreign sites (or a low density of such sites for cellular antigens) could only be contained by cell-mediated immunity, the decision criterion should ensure that such antigens normally only generate a cell-mediated response. It was encouraging that Pearson and Raffel had previously made the generalization, based upon observations, that antigens with few foreign sites could induce DTH but not antibody. In addition, although an antigen with many foreign sites could induce antibody formation, the dose of antigen administered was known to be critical. The administration of low doses of such antigens gave rise to exclusive cell-mediated immunity, and of higher doses to antibody production, following an initial, exclusive cell-mediated phase.

I proposed that the immune system uses the number of antigen-specific CD4 T cells that are present to gauge an antigen's degree of foreignness. This proposal assumes that there are no or relatively few CD4 T cells specific for self-antigens. This is an assumption with which most would now concur. I also assumed that the activation of (CD4) precursor T helper (pTh) cells requires other CD4 Th cells, according to the two signal model or more contemporary formulations (see A2, A3, A5-A8 above). The proposal that weak antigen-mediated CD4 T cell/ CD4 T cell interactions would lead to the generation of relatively few signal 2s, and to the activation of the pTh cell to express cell-mediated immunity (or give rise to Th1 cells in modern terminology) and that stronger interactions would lead to the generation of more signal 2s, and the generation of antibody (and of Th2 cells in modern terminology), seemed attractive. In this case, the threshold number of (helper T cell dependent) signal 2s required to generate cell-mediated (Th1 cells) and antibody (Th2 cells) responses would be different. This hypothesis is currently referred to as the Threshold Hypothesis for the nature of the decision criterion controlling the Th1/Th2 nature of the immune response. I argued in favor of the Threshold Hypothesis on the following grounds: (a) It made physiological sense (see (ii) immediately above). (b) It accounted for the fact that antigens with few foreign sites could only induce cell-mediated, Th1 responses. (c) It explained why



An explanation of how observaions on the conditions that lead to the induction of different classes of immunity are accounted for by The Threshold Hypothesis.

Figure 6 For explanation, see accompanying text

administration of antigens with many foreign sites, for which there were more CD4 T cells, could generate antibody responses and Th2 cells. (d) It explained why administration of low doses of such antigens with many foreign sites only induce cell-mediated, Th1 responses, as strong antigen-mediated interaction of the CD4 T cells cannot take place when antigen is very limiting; under these circumstances the signal 2s are not optimally generated or delivered. (e) It was known that antigen activated CD4 helper T cells. Thus antigen-mediated CD4 T cell/CD4 T cell interactions should improve with time, explaining how the immune response can evolve into an antibody, Th2 mode after passing through an exclusive cell-mediated, Th1 phase, see **NOTE** in Figure 6.

Finally, it was predicted that all cell interactions between antigen specific lymphocytes and involved in immune class regulation were mediated via the recognition of linked epitopes (see A5 for definition). This mechanism ensures the specificity of regulation of the class of immunity is specific and has important consequences, see B5 below.

B2a. Ramshaw, I.A., P.A. Bretscher and C.R. Parish. 1976. Regulation of the Immune Response I. Suppression of Delayed-type Hypersensitivity by T cells for Mice Expressing Humoral Immunity. European J Immunology. 6:674-79.

B2b. Ramshaw, I.A., P.A. Bretscher and C.R. Parish. 1977. Regulation of the Immune Response II. Repressor T Cells in Cyclophosphamide-induced Tolerant Mice. European Journal of Immunology. 7:180-185;

B2c. Ramshaw, I.A., I.F.C. McKenzie, P.A. Bretscher and C.R. Parish. 1977. Discrimination of Suppressor T Cells of Humoral and Cell-mediated Immunity by Anti-Ly and Anti-Ia Sera. Cellular Immunology.

Synopsis These three papers tested the predictions that cell-mediated and humoral immune deviation were mediated by TsAb and TsDTH cells respectively (see B1 for a definition of these cells) and established some of the characteristics of these cells.

Mice were induced to produce a strong antibody response to an antigen A. These mice could no longer be induced to express cell-mediated immunity in the form of DTH to a challenge of A that generated a DTH response in naïve mice. They were therefore in a state of humoral immune deviation. We showed, by transfer studies, that such mice contained in their spleen Ly1+Ly2- (CD4+ in current terms) T cells able to inhibit the generation of DTH in an antigen-specific manner. In addition, we showed that these TsDTH cells acted by linked recognition. Thus lymphocytes from mice made humorally immune to foreign haemocyanin (Hm) could inhibit the DTH response to horse red blood cells (HRBC), in the presence of Hm chemically attached to HRBC (Hm-HRBC), but not when Hm was present but not coupled to HRBC (i.e. in the presence of both HRBC and Hm coupled to mouse red blood cells (Hm-MRBC))**(B2a)**.

Mice were also induced to express strong cell-mediated immunity to an antigen C. These mice could no longer produce antibody following an antigen challenge that resulted in antibody production in naïve mice. They were therefore locked into a state of cell-mediated immunity, called a state of cell-mediated immune deviation (see B1). We showed, by transfer studies, that such mice contained in their spleen Ly1-Ly2+ (CD8+ in current terms) T cells able to inhibit the antibody response in an antigen-specific manner. In other words, TsAb cells do exist and have a CD4-CD8+ phenotype **(B2b)**.

These experiments defined two classes of regulatory T cell, TsAb and TsDTH cells, that differ in three characteristics. They are generated under different circumstances, they bear different surface markers **(B2c)**, and they inhibit different types of immune response, see Figure 7.

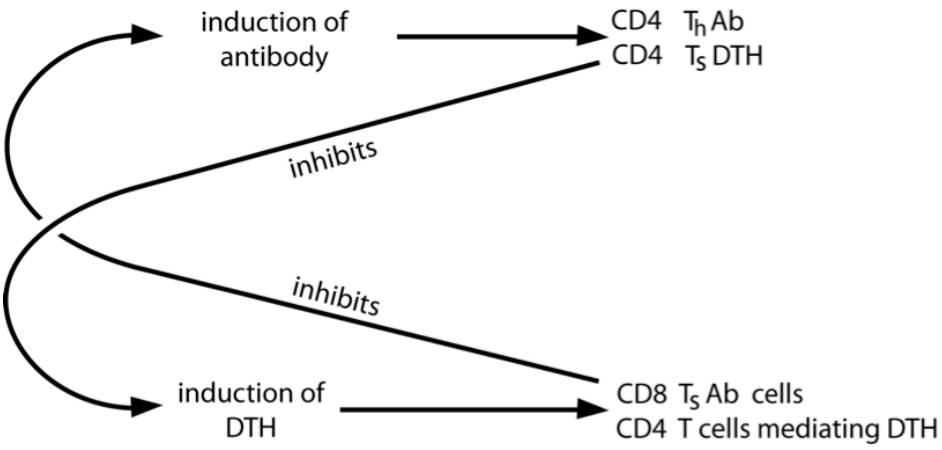


Figure 7 Summary of the experimental findings of B2a, B2b and B2c

We also examined the surface markers on cells mediating DTH. These were Ly1+Ly2- T cells, i.e. CD4 T cells in modern terms. We had therefore defined two classes of CD4 T cell, one that mediates DTH, and another, generated under conditions that result in antibody production, that does not express DTH but acts to inhibit the induction of DTH (B2c). It is natural to associate these two classes of CD4 T cell with the two types of CD4 T cell clones, Th1 and Th2, characterized considerably later by Mosmann and Coffman, on the basis of the lymphokine spectrum they express and the biological activities they mediate.

In the contemporary context, it seems to be almost universally held that Th1 cells inhibit the generation of Th2 cells and vice versa. I feel the experimental basis for the inhibition of Th2 responses by Th1 cells not to be impeccable. This inference comes from fairly artificial systems. Indeed, some of the evidence that led to a Theory of Immune Class Regulation was that the three conditions under which exclusive DTH is induced are just those giving rise to the generation of CD8 TsAb cells (see B1).

However, these experiments by others were done in diverse systems. No one, as far as I know, had looked at a situation where both DTH was induced and the animal was unresponsive for the induction of antibody, and had tried to define the nature of the TsAb cells responsible, until we did so. We found the TsAb to be CD8 T cells. I feel this question of whether CD4 or CD8 T cells, or both cell types, inhibit the generation of Th2 cells, is still unresolved (see B4).

B3a Bretscher, P.A. 1979. In-vitro Induction of delayed-type hypersensitivity. European Journal of Immunology. 9:311-16

B3b Bretscher, P.A. 1983. In Vitro Analysis of the Cellular Interactions Between Unprimed Lymphocytes Responsible for Determining the Class of Response an Antigen Induces: Specific T Cells Switch a Cell-mediated Response to a Humoral One. Journal of Immunology. 131:1103-7

B3c. Bretscher, P.A. 1983. Regulation of the Immune Response Induced by Antigen. I. Specific T Cells Swith the In-vivo Response from a Cell-mediated to a Humoral Mode. Cellular Immunology. 81:345-56.

Synopsis We wished to develop in vitro and in vivo systems in which we could generate both primary cell-mediated and antibody responses. Such systems should allow one to alter various experimental parameters to determine how such parameters affect the class of immunity generated. Such systems should allow one to test various hypotheses as to the nature of the decision criterion controlling the cell-mediated/humoral (or Th1/Th2) nature of the immune response.

We first found conditions *in vitro* under which we could generate primary DTH responses, involving a medium density of unprimed spleen cells. We then found that, holding the amount of antigen per culture well constant, that low densities of unprimed spleen cells did not support the generation, during six days of culture, of cells mediating DTH or of cells producing antibody. Similar cultures but consisting of a medium density of unprimed spleen cells supported the generation of DTH-mediating T cells but few cells producing antibody. Similar cultures but consisting of a high density of unprimed spleen cells supported substantial antibody production but not substantial generation of DTH mediating cells. In other experiments, lethally irradiated mice were given a constant amount of antigen and reconstituted with different numbers of unprimed spleen cells. Mice given a medium number of spleen cells supported the generation of DTH-mediating cells, and little antibody production. Mice reconstituted with greater numbers of unprimed spleen cells supported strong IgG antibody responses and the generation of DTH-mediating cells was poor. The in vitro and in vivo findings were remarkably parallel, giving us confidence in the validity of the in vitro system. Higher densities or numbers of unprimed spleen cells were required to generate antibody than DTH responses. Further observations showed that it was the greater number of T cells in

higher densities/numbers of unprimed spleen cells, rather than the greater number of APC, that was required to obtain antibody production and a decreased generation of DTH-mediating cells. We shall return to this system when discussing a more recent study employing more sensitive techniques (B6 below), and discuss its relevance to critical testing of the Threshold Hypothesis (see B1 above).

B4a S.A. LeCercq and P.A. Bretscher. 1987. T Cells Expressing Delayed-type Hyper-sensitivity can be Derived from a Humorally Immune Lymphocyte Population. Eur. J. Immunol. 17, 949-954.

B4b Tuttosi, S. and P.A. Bretscher. 1992. Antigen-specific CD8+ T Cells Switch the Immune Response Induced by Antigen from an IgG to a Cell-mediated Mode. J. Immunol. 148:397-403.

Synosis In vivo experiments show that immune responses can become locked into humoral or cell-mediated modes (see B1). These "locks" are mediated, at least in part, by TsAb and TsDTH cells (see B2). If we understand these locks, we may be able to pick them. In addition, effector cells of a given type are likely to be in a dynamic state during the course of an on-going immune response, dying and being regenerated. If we can find conditions inimical to their regeneration, we may be able to get rid of them. The potential clinical importance of such modulation is evident in cancer and in some infectious diseases, as discussed later, see C7-C9. We explored in these papers the possibility of modulating responses from an antibody (now known to be a mixed Th1/Th2 response) to a Th1 mode.

We cultured humorally immune spleen cells under various conditions and, after a few days, assessed the production of antibody and the presence of DTH mediating cells. The in vivo immunized spleen cells were producing much antibody and expressing very little DTH at initiation of culture. Little immunological reactivity was sustained by these cells if the spleen cells were cultured without antigen. Under all conditions realizable in vitro, antibody production decreased and DTH reactivity appeared after several days of culture in the presence of antigen **(B4a)**. Restimulation under certain conditions resulted in the virtually complete absence of antibody production and the expression of massive DTH. We showed that this cell population contains CD8 T cells that can switch in vivo immune responses from an antibody to a cell-mediated mode, i.e. CD8 TsAb cells **(B4b)**. We conclude that mixed Th1/Th2 responses can be readily modulated into a predominant Th1 mode. These findings influenced our therapeutic strategy in the murine model of cutaneous leishmaniasis (see C7) and have implications for treatment of AIDS, as discussed in my two immunology-books.

B5. N. Ismail and P.A. Bretscher, 1999. The Th1/Th2 nature of concurrent immune responses to unrelated antigens can be independent. J. Immunol. 163:4842-4850.

Synosis It is recognized that the Th1/Th2 nature of the immune response generated against many pathogens is critical to whether containment of the pathogen is achieved. It also seems plausible that concurrent, subclinical infections by different pathogens often occur under natural conditions. Given these likelihoods, it would seem important that the Th1/Th2 nature of concurrent immune responses are independently determined under most circumstances. For example, it would seem physiologically detrimental if a Th2 response to a subclinical infection of a helminth deviated an immune response to *Mycobacterium tuberculosis* towards the Th2 pole, thereby resulting in tuberculosis. We test here the "Independence Hypothesis". This hypothesis postulates that the Th1/Th2 nature of simultaneous responses to non-cross-reacting antigens, even when generated in the same secondary lymphoid organ, can be independently determined. We show that a low dose of xenogeneic red blood cells (XRBC) given by itself i.v. generates an exclusive Th1 splenic response; the administration of a high dose of a non-cross-reacting XRBC by itself generates a predominant Th2 splenic response. Mice immunized with both antigens simultaneously mount splenic responses indistinguishable from that generated in singly immunized mice. This provides support for the Independence Hypothesis.

B6 Testing predictions of the Threshold Hypothesis

B6a. N. Ismail and Peter Bretscher, 2001, More antigen-dependent CD+ Tcell/CD4+ T cell Interactions are required for the Primary Generation of Th2 than of Th1 cells, European Journal of Immunology, 31:1765-1771.

B6b Ismail N, Basten A, Briscoe H, Bretscher PA. Increasing the foreignness of an antigen, by coupling a second and foreign antigen to it, increases the T helper type 2 component of the immune response to the first antigen. *Immunology*. (2005) 115:34–41.

Synopsis The experiments reported represent stringent tests of the Threshold Hypothesis (B1).

We employed lethally irradiated mice reconstituted with various populations of unprimed spleen cells and challenged them with different doses of antigen (see B3c). We then measured at day 6 after irradiation, reconstitution and immunization, the generation of antibody producing cells, of antigen-specific Th1, IFN-γ and of antigen-specific Th2, IL-4 producing CD4 T cells, employing an ELISPOT assay for enumerating single, antigen-specific cytokine producing cells (see A10a).

We initially employed a substantial dose of antigen that generates a strong IgG antibody response and substantial Th2 cells in intact mice. Irradiated mice reconstituted with a medium number of unprimed spleen cells generated little antibody and mounted a predominant Th1 response upon immunization, whereas mice reconstituted with a large

number of unprimed spleen cells produced much IgG antibody and substantial numbers of Th2 cells were generated. Clearly a higher number of unprimed spleen cells are required to generate Th2 cells and antibody producing cells than are required to generate Th1 cells. Further experiments showed that it is the greater number of CD4 T cells in a large than in a medium number of spleen cells that are required to obtain IgG antibody production and the generation of Th2 cells. There was no detectable role for CD8 T cells or for antigen presenting cells. Moreover, the number of CD4 T cells and the antigen dose were interdependent variables in determining whether Th2 cells were generated. For example, a slight decrease in antigen dose, that by itself would result in a loss of the generation of Th2 cells, could be compensated for by increasing the number of CD4 T cells, so that Th2 cells were still generated. These observations are expected if more *antigen-mediated* CD4 T cell/CD4 T cell interactions are required for the generation of Th2 than for the generation of Th1 cells. These observations are highly characteristic predictions of the Threshold Hypothesis, and appear to support its plausibility **(B6a)**.

We also decided to test a critical prediction of the Threshold Hypothesis in intact mice. Basten and Goodnow had made different mice transgenic for the antigen hen egg lysozyme (HEL). In collaboration with Tony Basten, we employed one of their transgenic mice known to be tolerant to HEL at the T cell level. We immunized normal and transgenic with a low dose of foreign red blood cells (FRBC), chosen to generate a Th1 response. We coupled HEL to the FRBC and immunized normal and transgenic mice with a low dose of the conjugated-FRBC. HEL transgenic mice generated a Th1 response to the FRBC, upon such immunization, but the response of normal mice had a greater Th2 component. The responses of normal and transgenic mice were identical on challenge with a low dose of FRBC to which ovalbumin had been conjugated, both generating a response with an increased Th2 component **(B6b)**. It thus appears that the Th2 component of the response to the FRBC can be increased in normal mice by immunizing with the FRBC-HEL conjugate, making the antigen more foreign, but this increase is not seen in the response generated in HEL transgenic mice, tolerant to HEL at the T cell level.

B7 Rudulier CD, McKinstry KK, Al-Yassin GA, Kroeger DR, Bretscher PA. The Number of Responding CD4 T Cells and the Dose of Antigen Conjointly Determine the Th1/Th2 Phenotype by Modulating B7/CD28 Interactions (2014). The Journal of Immunology. 192: 5140-50

This paper describes critical predictions of the Threshold Hypothesis in both in vivo and in vitro systems. We show that TcR transgenic CD4 T cells specific for an antigen R will modulate the *in vivo* response to the antigen Q, in the presence of the Q-R conjugate, but not in the presence of Q and R as separate molecules. We showed that the same Q-specific CD4 T cells could facilitate the generation of R-specific Th1 cells with a small, limiting dose of the conjugate Q-R. The very same Q-specific CD4 T cells could modulate a Th1 response towards a Th2 mode when the dose of the Q-R conjugate was chosen to generate a Th1 response in the absence of the Q-specific

CD4 T cells.

The in vitro experiments were carried out with TcR transgenic CD4 T cells in a system based on the study with polyclonal CD4 T cells (B3b). We found, in the presence of a standard number of APC, that different numbers of transgenic CD4 T cells generated different effectors, lower numbers of CD4 T cells giving rise to Th1 cells and higher numbers of CD4 T cells to predominant Th2 responses. The Th1/Th2 phenotype of the response also depended in the expected way on antigen dose. We made the hypothesis that the immune system "counted" the CD4 T cells present by a mechanism involving the up-regulation of CoS molecules as CD4 T cells interacted with them. Our experiments support the idea that a number of potential costimulatory molecules and their counter receptors are not involved, and that the B7/CD28 costimulatory signals are involved.

B8 Reviews on how the Th1/Th2 phenotype of the response is determined

B8a Peter Bretscher (2014) On the mechanism determining the TH1/TH2 phenotype of an immune response, and its pertinence to strategies for the prevention, and treatment, of certain infectious diseases. Scand J Immunol. 2014;79(6):361-76.

B8b Peter Bretscher (2019) On analyzing how the Th1/Th2 phenotype of an immune response is determined: classical observations must not be ignored, Frontiers in Immunology

I was motivated to write these two papers in view of the fact that virtually all analysis, on the mechanism by which the Th1/Th2 is determined and carried out by others during the last three decades, were in the context of two frameworks that I find implausible: The Cytokine Milieu Hypothesis and the pathogen-associated molecular pattern (PAMP) Hypothesis. These two hypotheses are inconsistent with many quantitative observations on the variables of immunization that affect the Th1/Th2 phenotype of the response, as I argue in these papers. These quantitative variables are those that are accounted for by the Threshold Hypothesis **(B1a and B1b)**. Moreover, these two frameworks are employed in trying to develop strategies of prevention and treatment of many clinical situations, related to infectious disease, allergies, autoimmunity and cancer. I illustrate in this paper how the Threshold Hypothesis can be employed to develop such strategies. Such strategies are also described under Area C below.

Area C Vaccination/modulation of the immune response against intracellular pathogens causing chronic disease: studies in humans and in model systems

Introductory Background

Vaccination against polio and small pox is extremely effective. Immunologists believe they know why. The immune response that ensues following natural infection is larger and more rapid in vaccinated than in naïve individuals. This increased speed and intensity of the immune response provides the vaccinated individual with the edge in the race between making an immune response that contains the infection and the unrestrained multiplication of the pathogen that leads to disease. The effectiveness of vaccination against small pox and polio contrasts with our inability to vaccinate effectively against the bacterium, *Mycobacterium tuberculosis*, that can cause tuberculosis, or the virus, HIV-1, that can cause AIDS. Medical observations provide clues as to why this might be so. Some individuals that are infected by these pathogens do not become ill. An examination of their immune status shows that they often have a predominant cell-mediated, Th1 response, often with the production of little antibody. In contrast, the immune response of patients often has a substantial Th2 component. Is this fortuitous or significant? The only way of telling is to find a way of vaccinating individuals in a manner that guarantees a strong and predominant Th1 response upon natural infection, and ascertaining whether indeed this results in protection. Such experiments have been successfully carried out in mice. These experiments suggest it is not just the size but the *type* of response that is critical in determining whether an immune response is effective.

The murine model of human cutaneous leishmaniasis has become the most developed animal model for examining the regulation of immunity against intracellular pathogens that can cause chronic disease. The hope is that we shall uncover general principles applicable in diverse systems.

Leishmania parasites multiply inside macrophages. Infection of some strains of mice with a million parasites results in an initial increase in parasite burden, the generation of a stable and predominant Th1 response, and containment of the parasite. The parasite does not spread to sites distal from the initial site of infection. Such mice are called “resistant”. Mice of other strains, similarly infected, produce in time a predominant Th2 response against the parasite. This response is associated with increasing parasite burden and the spread of the parasite to sites distal from the site of initial infection and, in the long run, death. Such mice are called “susceptible”. Is the association between a Th1 response and resistance, and between a Th2 response and susceptibility, merely accidental or causal? Various manipulations at the time of infection of susceptible mice result in a change in the long term nature of the immune response, from a Th2 to a Th1 mode, and these manipulations also allow the mice to resist the infection. Manipulations

to resistant mice at the time of infection, that change the response from a Th1 to Th2 mode, result in susceptibility. These observations provide compelling evidence that the correlations between a Th1 response and resistance, and between a Th2 response and susceptibility, are not accidental but meaningful.

C1 Bretscher, P.A., G. Wei, J.N. Menon and H. Bielefeldt-Ohman. 1992. Establishment of Stable Cell Mediated Immunity Makes "Susceptible" Mice Resistant to *Leishmania major*, Science, 13:342.

Menon, J.N. and P.A. Bretscher. 1996. Characterization of the immunological memory state generated in mice susceptible to *Leishmania major* following exposure to low doses of *L. major* and resulting in resistance to a normally pathogenic challenge. Eur. J. Immunol. 26:243-49.

Synopsis BALB/c mice are susceptible to infection with a million leishmania parasites. Such an infection generates a small and transient DTH response, and parasites grow in number and spread as parasite-specific Th2 are generated with an ever decreasing presence of parasite-specific Th1 cells. In contrast, infection of the same mice with about a thousand parasites results in a prolonged and sustained Th1, DTH response, little antibody production, and containment of the parasite. Lesions, if they form at all, are small, transient and the mice heal. We examined whether we could establish cell-mediated immune deviation by this low dose infection, in a manner reminiscent to that developed by Parish employing a protein antigen in rats. Infection of mice with a million parasites, that had been infected with a low number of parasites about two months previously, reliably resulted in remarkable resistance. Pre-exposure to a low dose infection resulted in a modulation of the response, following infection with a million parasites, from a Th2 to a Th1 mode. Lesion formation was transient. Low dose infection thus constitutes effective vaccination. These studies show that “susceptibility” is not an absolute trait, but one conditional on parasite dose. Also, “susceptibility” is also not absolute in the sense that it is modifyable, in this case by pre-exposure to a low dose infection. Conditionality and modifyability are essential attributes of susceptibility if vaccination is to be feasible. We refer to the process of vaccinating in a manner that guarantees a Th1 response to subsequent infections as Th1 imprinting.

C2 Bretscher, P.A. 1992. A Strategy to Improve the Efficacy of Vaccination against Tuberculosis and Leprosy, Immunology Today, 9:342-3454

Synosis This paper reviews BCG vaccination trials against tuberculosis, and proposes, in view of our findings in the mouse model of cutaneous leishmaniasis (see C1 above), that lower doses of BCG may be more efficacious in preventing tuberculosis and leprosy than the standard dose of BCG currently employed. Our studies in murine leishmaniasis had shown that infection with a low number of parasites could generate a Th1 response and a Th1 imprint upon the immune system, guaranteeing a protective,

Th1 response upon infection with a high of parasites that was lethal in normal mice. A recognized and major problem in developing a standard vaccination protocol that is universally effective is due to the genetic diversity of the vaccinated population. This diversity means that the immune response to a standard vaccination protocol will generally be different in different people. A strategy to overcome this problem is suggested. It is proposed that vaccination should be carried out with a very low dose of BCG that does not generate a Th2 response in any member of the population. It will then either generate a Th1 response and a Th1 imprint, or be below the threshold required to do so. In the latter case, the BCG bacteria, being alive, will grow unimpeded, until they reach the threshold level needed to induce a Th1 response. They should then induce such a response and a Th1 imprint. This strategy is supported by the study described in C4 below.

C3 Salk, J., Bretscher, P.A., Salk, P., Clerici, M., Shearer, G.M. 1993. A Strategy for Prophylactic Vaccination against HIV. Science 260:1270-127; Salk, J., Bretscher, P.A., Salk, P., Clerici, M., Shearer, G.M. 1993. Protection from HIV infection or AIDS? Science 262:1075-76, under "Technical Comments".

Synopsis Discusses the possibility of developing an effective AIDS vaccine by Th1 imprinting and describes evidence supporting the plausibility of this approach.

C4 Menon, J.N. and P.A. Bretscher, 1998. Parasite dose determines the Th1/Th2 nature of the response to *Leishmania major* independently of infection route, strain of host or of parasite. Eur. J. Immunology 28:4020-4028.

Synopsis We examined the dose dependence of the Th1Th2 phenotype of the immune response under a variety of conditions. We examined different parasite strains, three different strains of mice and two different routes of infection (subcutaneous and intravenous). We found in all cases studied that we could define a “transition number” of parasites, t_n. Infection with a number of parasites below t_n resulted in a stable Th1 response; with a number above t_n in a Th2 response and progressive disease. The value of t_n could vary dramatically between hosts. We found a case where the value of n^t for the same parasite strain, same site of infection, but different hosts, differed by about a million fold. These studies strongly support the generality of the dependence of the Th1/Th nature of the response upon antigen dose or parasite number. This is potentially very important as it might provide the means of achieving universally effective vaccination by a standard vaccination strategy in a genetically diverse population (see C2 above).

C5 Power, C., G. Wei and P.A. Bretscher. 1998. Mycobacterial Dose Defines the Th1/Th2 Nature of the Immune Response Independently of Whether Immunization is by the Intravenous, Subcutaneous or Intradermal Route. Infection and Immunity, 66:5743

Synopsis Shows that the Th1/Th2 nature of the response in BALB/c mice to mycobacteria depends upon the mycobacterial dose independently of whether infection is by the intradermal, subcutaneous or intravenous route. Lower doses induce predominant Th1 responses, and higher doses mixed Th1/Th2 responses.

C6 Jude E. Uzzona, Guojian Wei, Dean Yurkowski, and Peter Bretscher, 2001. Immune elimination of *Leishmania major* in mice: implications for immune memory, vaccination and reactivation disease, J. Immunol. 167:6967-6974

Synopsis Provides evidence that BALB/c mice, infected with a low number of parasites, can generate a predominant Th1 response and can sometimes eliminate the parasite, previously thought never to occur. We also describe a reliable system in which elimination can be conclusively demonstrated and the consequences of such elimination examined. Elimination results both in loss of resistance and the disappearance of parasite-specific lymphocytes, as assessed by limiting dilution analysis. These observations show, in a very natural situation, the dramatic role of antigen in maintaining immunological memory. This has obvious implications for vaccination strategies, and for the possibility of controlling reactivation disease. (Reactivation disease occurs when a chronic, subclinical infection, held in check by the immune system, erupts due to some immunosuppressive events. Reactivation tuberculosis, that can occur in AIDS patients, could be prevented if ways were found by which the immune system could be conditioned to eliminate *M. tuberculosis*).

C7 Jude E. Uzonna, and P.A. Bretscher, 2001, Anti-IL4 antibody therapy causes regression of chronic lesions caused by medium-dose *Leishmania major* infection, European Journal of Immunology, 31:3175-3184 (2001)

Synopsis BALB/c mice are susceptible to an infection with a million parasites. Several manoevres, carried out at the time of this infection, result in resistance. These include treatment with anti-IL-4 antibody, with anti-CD4 antibody and administering IL-12. These manoevres are ineffective once these infections become established. The conventional wisdom is that such simple manoevres do not provide models for how human disease might be treated.

Previous work shows that mixed Th1/Th2 responses may be readily modulated to a predominant Th1 mode (see B4). In addition, the mouse model of infecting BALB/c mice with a million leishmania parasites may not realistically reflect much human disease. The immune response in this particular mouse model becomes fairly rapidly of a very dominant Th2 phenotype, whereas responses may well be less polarised in much human disease. We therefore employed infection of BALB/c mice with about 10,000 leishmania parasites, which we knew (C1 above) often resulted in large, stable lesions, associated with a mixed Th1/Th2 response. We refer to such a diseased state as a state of borderline leishmaniasis. We felt these immune states and lesions might better

correspond to much human disease. We found borderline leishmaniasis readily treatable with simple manoevres, such as administration of neutralising anti-IL-4 antibody or of anti-CD4 antibody. Such treatment resulted in a complete resolution of very large lesions, and also a modulation of the immune response to a predominant Th1 mode. Recovered mice were completely resistant to a challenge of a million parasites that is progressive in normal mice. This acquisition of resistance seems analogous to what happens in patients with visceral leishmaniasis who are treated with anti-parasite drugs (see C8).

C8 A. Hailu, J.N. Menon, N. Berhe, L. Gedamu, T. Hassard, P.A. Karger, J. Olobo, A. Senthilsen, and P. A. Bretscher, 2001, Distinct immunity in visceral leishmaniasis patients from that in healthy contacts and drug-cured people: implications for the mechanism underlying drug cure, J. Infectious Diseases, 184:112-115

Synopsis We examined the levels of anti-leishmania parasite antibody belonging to different IgG subclasses in (a) normal individuals (b) patients, (c) healthy contacts and (d) drug-cured individuals. Dramatic differences are readily seen between the antibody levels of patients and healthy contacts. Patients had higher levels of IgG1, IgG3 and IgG4 antibodies, but similar levels of IgG2 antibody. Most interestingly, the immune state of drug cured individuals and healthy contacts were indistinguishable. This explains the known resistance of most individuals, that have undergone anti-parasite drug therapy, to re-infection. Our observations demonstrate that human immune responses can be modulated. We argue that modulation of the response from a mixed Th1/Th2 to predominant Th1 mode probably occurs due to a lowering of the antigen load following drug treatment and parasite death.

C9. P.A. Bretscher, N. Ismail, J.N. Menon, C.A. Power,. J. Uzonna and G. Wei, 2001. Vaccination against and treatment of tuberculosis, the leishmaniases and AIDS: perspectives from basic immunology and immunity to chronic intracellular infections. Cellular and Molecular Life Sciences, 58: 1879-1896

Synopsis We outline our view on how concepts derived from basic studies might have a beneficial impact upon strategies of vaccination. This is in the context of intracellular pathogens that can cause chronic disease. We try to justify the concept of “coherence”, as follows. Suppose we represent the proteins of a parasite as Q, giving rise to q1,q2,q3,….qn peptides recognised by CD4 T cells in the context of host class II MHC molecules. The idea of coherence reflects the fact that the Th1/Th2 nature of the response to q1, q2. ….qn are not independently determined but are interdependent. The response to all the peptides tends to be predominantly Th1, or predominantly Th2, or predominantly mixed under a given set of circumstances. We discuss regulatory mechanisms that account for coherence. It is argued that we can ensure effective protection by ensuring that certain global features of the immune response are appropriate, such as its strength and its Th1/Th2 nature, without the need for defining protective antigens and the detailed specificity of protective T cells. We discuss in

general terms some of our then unpublished findings in tuberculosis and our unpublished findings in leprosy. We outline our favored strategies in preventing and treating tuberculosis, AIDS and the leishmaniases.

C10a P.A. Bretscher , J.N. Menon, C. Power, J. Uzonna, and G. Wei, 2001, A case for neonatal, low dose BCG vaccination, Scandinavian Journal of Infectious Diseases, 33:253-257

C10b TG Kiros, CA Power, G Wei, and P Bretscher, 2010, Immunization of newborn and adult mice with low numbers of BCG leads to Th1 respnses, Th1 imprints and enhanced protection on BCG challenge. Immunotherapy, 2: 25-35

Synopsis C10a gives what was then the most recent and complete presentation of the case for a neonatal, low dose BCG vaccination trial against tuberculosis. We tested this strategy (C10b). At the time of these studies, a leading investigator, in discussing immunization of mice with BCG, called immunization with a million colony forming units (cfu) of BCG as immunization with a low number of BCG. We show in this paper that immunization of very young mice with 33 cfu of BCG generates very strong Th1 responses and the best Th1 imprints! Buddle and colleagues have experimentally found a way to protect cattle against experimental tuberculosis. Part of the secret of their success was that they reduced the vaccination dose by about 10^6 fold!

C11 A hypothesis for the existence of two types of tuberculosis, reflecting two distinct types of immune failure to control the pathogen, based upon prevalence of mycobacterium-specific IgG subclasses, 2018, J Menon, VH Hoeppner, A Judd, CA Power, PA Bretscher, Scand J Immunol e12665

Tuberculosis (TB) is among the greatest killers of all infectious diseases worldwide. Although many think Th1 responses are protective, much of the pathology associated with TB is caused by lung granulomas, also caused in large part by a Th1 response to bacterial antigens. These facts illustrate a major quandary of TB research: what is different about the Th1 response in healthy infected individuals and in patients? We need to know what immunological parameters discriminate between responses in patients and in healthy infected individuals if we are to rationally design vaccines against TB. In addition, these correlates are important when considering immunotherapy of this infectious disease.

We approached this question by measuring the relative prevalence of different subclasses of IgG antibodies, among those specific for mycobacterial antigens, in patients and healthy infected individuals. We calculated the ratio of IgG1 to IgG2 antibodies. We found that this ratio varied about a thousand fold among healthy infected (0.001 to 1) and about hundred, thousand fold among patients (0.001 to 100). It was evident that patients with IgG1 to IgG2 ratios between about 1 to 100 had a greater Th2 component to their anti-mycobacterial-specific immune response than patients and

healthy infected individuals with lower ratios (0.001 to 1). This led us to suppose that the immune response of these patients had a down-regulated Th1 response, and so could not contain the pathogen. We refer these patients as having type 2 tuberculosis. Why might there be other patients with qualitatively similar responses to those of healthy infected individuals?

We were very impressed by our findings that the transition number for the response to *Leishmania major* varied over almost a million-fold range **(C4)**. We argue that if there is anything like a similar variation in humans, in their response to *Mycobacterium tuberculosis*, the nature of the response will be widely different upon exposure to an infectious dose of *M tuberculosis*. We argue in this paper that people with a very high transition number will only make a response to *M tuberculosis* once the bacterial burden has become considerable. Such individuals, having a greater bacterial burden, would also need a much stronger Th1 response to win the war against the pathogen. We thus propose that patients, with IgG1 to IgG2 ratios between 0.001 to 1, have a similar response to healthy infected individuals, but the type I TB patients have too small a Th1 response to contain the infection. We discuss how this idea allows several paradoxes concerning immunity to *M tuberculosis* to be resolved. There are implications of this hypothesis for both vaccination and treatment.

Area D: Tumor Immunology

D1 The Th2 Skewing Hypothesis of tumor escape: implications for vaccination against and immunotherapy of cancer

D1a Hamilton, D. H. and P. A. Bretscher (2008). "Different immune correlates associated with tumor progression and regression: implications for prevention and treatment of cancer." Cancer Immunol Immunother 57(8): 1125-36.

D1b Hamilton, D. H. and P. A. Bretscher (2008). The commonality in the regulation of the immune response to most tumors: the prevalence of immune class regulatio as a tumor escape mechanism. Cancer Therapy 6:745-754

D1c Duane Hamilton, Nahed Ismail, David Kroeger, Christopher Rudulier, Peter Bretscher(2009). Macroimmunology and immunotherapy of cancer. Immunotherapy 1:367-383

Synopsis Recent reviews of the relationship between cancers and the immune system provide a somewhat dismal prospect for vaccination against and immunotherapy of cancer. This is because many and diverse mechanisms of tumor escape from the surveillance of the immune system have been described. How can we provide protection against all these diverse escape mechanisms?

We felt and feel differently about the prospects for vaccination and immunotherapy. There are clearly diverse mechanisms of escape, but we strongly argue that in mouse systems cell-mediated, Th1, cytotoxic T lymphocyte immunity is protective, and tumor progression correlates very frequently with the generation of a Th2 component of the anti-tumor response. We tested this Th2-skewing hypothesis in two different and well studied murine tumor systems and found it held **(D1a)**. We discuss the significance of this finding for understanding the immunological basis of various empirical therapies and for developing effective vaccnation and immunotherapy. We discuss the possibility that a similar situation holds in human cancer **(D1b and D1c)**.

Area E: Miscellaneous

E1 Bretscher, P.A. 1987. Signs and Meaning in the Study of the Immune and Higher Nervous Systems, in "The Semiotics of Cellular Communication in the Immune System". E.E. Sercarz, F. Celada, N.A. Mitchison & T. Tada, Eds., NATO ASI Series, Springer-Verlag, Publ. pp 281-304.

E2 Bretscher, P.A. 1988. Decision Criteria and Coherence in the Regulation of the Immune Response, in Theoretical Immunology, A.L. Perelson, Editor, Part I, 295-307, Addison-Wesley Publishing Co.

Synopsis A discussion of how simple observations imply that there must be sophisticated interactions between antigen-specific lymphocytes. lymphocytes do not behave as independent entities, but as well regulated members of a society with rules. I argue that the behavior of lymphocyte populations must reflect the existence of more interactions between lymphocytes that usually envisaged.

E3 Bretscher, P.A. and C. Havele. 1992. Cyclosporine A can Switch the Immune Response Induced by Antigen from a Humoral to a Cell-mediated Mode. European Journal of Immunology. 22:349-355.

Synopsis We show that the very same dose of cyclosporin A (CsA) can, under different circumstances, have very different and what at first appears to be contradictory effects. A given concentration of CsA can *inhibit* the generation of cell-mediated immunity in the form of delayed type hypersensitivity (DTH). The very same concentration of CsA can inhibit an antibody response and *increase* the induction of DTH. These diverse effects of the same concentration of CsA on the generation of cell-mediated immunity can be understood if CsA inhibits the generation of eTh, and if the generation of antibody responses are more helper T cell dependent than is the generation of cell-mediated immunity in the form of DTH.