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SPECIFICITY AND DEGENERACY IN ANTIGEN RECOGNITION: Yin and Yang in the Immune System

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■ **Abstract** One of the hallmarks of the immune system is specificity, a concept based on innumerable observations that antibodies react with the substance that elicited their production and only a few other structurally similar substances. The study of T cells has begun to suggest, however, that in responses mediated by their antibody-like receptors (T cell receptor or TCR) an individual T cell, expressing a singular TCR, can discriminate as exquisitely among antigens as the most specific antibodies but also exhibit "degeneracy": i.e., it can react with many disparate antigens (peptide-MHC complexes). An explanation for this duality (specificity and degeneracy) can be found in (i) the powerful amplifying signal transduction cascades that allow a T cell to respond to the stable engagement of very few TCR molecules, initially perhaps only one or two out of around 100,000 per cell, by their natural ligands (peptide-MHC complexes or epitopes on antigen-presenting cells—or APC) and (ii) the inverse relationship between TCR affinity for epitopes and epitope density (the number of copies of an epitope per APC). Older observations on the excess of total globulin production over specific antibody production in response to conventional immunization procedures suggest that B cells also exhibit degeneracy, as well as specificity. These views are developed against a backdrop describing how the author became interested in the immune system and has pursued that interest.

> "... a concept of science drawn from ... [textbooks] ... is no more likely to fit the enterprise that produced them than an image of a national culture drawn from a tourist brochure."
>
> Thomas Kuhn, *Structure Of Scientific Revolutions*

INTRODUCTION

Sometime around 1960 Rene Dubos, a distinguished microbiologist, visited Washington University School of Medicine in St. Louis to talk about special bacterial cultures as sources of novel enzymes. I was his official host and expected

us to discuss similarities and differences between adaptive enzymes, as they were then called, and antibodies. Instead, he kept wanting to know how I had become interested in immunology. I remember being much annoyed by his persistent interest and ignored it. But I never forgot it and I address it now, not because the answer is especially interesting, but because his question was reasonable, for when my interests had developed there were virtually no textbooks or courses in immunology, and only a few days were devoted to it in medical school curricula. The attempt to answer the question also provides a convenient starting point for this anecdote-driven approach to the principal theme of this chapter—how the idea of specificity in immune reactions, grounded in a century of experience with antibodies, is likely to be significantly modulated by evidence that a T cell, expressing a singular antibody-like receptor, can respond to many disparate antigens (generally, short peptides associated with major histocompatibility complex (MHC) proteins.

EARLY VIEWS AND ACTIVITIES

To begin with some personal notes, it seems to me that my early views and activities were much influenced by two circumstances: First, I grew up in a large Jewish community in Brooklyn, a part of New York City that at the time (the 1920s) seemed suburban. My father, as a 12-year-old accompanied only by his younger brother, had migrated from what was then part of the Austro-Hungarian Empire to New York in 1895. They joined my grandfather, who had migrated several years before, and my mother, who, as a child, had similarly arrived in the United States with her parents at about the same time from eastern Europe. With that background it should not be surprising that I grew up with a sense that antisemitism in the world around us was pervasive. Though certainly not as overt as in eastern Europe, in the United States it was nevertheless regarded as a fact of life, like birth and death. When chemistry in high school was the only subject I found interesting and I expressed an interest in becoming a chemist or chemical engineer, my father argued that this was not a realistic choice because of dismal employment possibilities: Large companies in the chemicals industry did not hire Jews. In medicine, however, a somewhat related field, one's destiny was in one's own hands. So, because my father was persuasive and I was not yet 16 when I entered college, I enrolled as a premedical student.

The other circumstance that may be worth mentioning is that while in high school I was suspected of having pulmonary tuberculosis. The diagnosis became firmer after one year of college and led to a year's enforced bed-rest. I read extensively and remember being particularly affected by Sinclair Lewis's *Arrowsmith*, with its idealistic physician-scientist as the main protagonist, and by Darwin's *Origin of Species*, with its powerful but simple idea expressed in chapter after chapter of magnificent prose. (But Darwin's *On the Descent of Man* I found impenetrable and never finished.)

At New York University (NYU), as an undergraduate, I happened to attend a seminar on the synthesis of some epinephrine-like molecules and their physiologic effects. The speaker, a graduate student in organic chemistry named Morris Ziff, who later became a distinguished rheumatologist, described how small changes in structure, such as shifting an OH group from one carbon in a benzene ring to an adjacent carbon, led to dramatic effects on a cat's blood pressure. The fascination of seeing, as an impressionable teenager, the connection between molecular structure and biologic function made a deep impression and must have left me especially receptive to the chemically oriented immunology I would shortly after encounter in papers from the Landsteiner and Pauling laboratories.

The medical school I attended from 1939 to 1943 (New York University, usually called Bellevue after its great teaching hospital) bore no resemblance to its counterparts in the following decades or now. Teaching in the clinical departments depended upon a part-time faculty who earned their livelihood in solo private practice. The preclinical departments were remarkably small. For instance, the biochemistry department had, I think, four faculty members and about the same number of graduate students; grants to support research were pitifully meager. There was nevertheless a sense that serious research was being carried out, especially in certain fields, among them infectious diseases. The latter was marked later by the triumphs of Jonas Salk and Albert Sabin (both of whom had graduated a few years previously) in developing the vaccines against poliovirus that may well now be on the way to eliminating this pathogen from the world.

In contrast, the research environment at Columbia University's College of Physicians and Surgeons, where I subsequently served as a resident in pathology, was vastly larger and more impressive. It listed a large and illustrious faculty, especially in biochemistry, many of them Jewish refugees from Nazi Germany. It had an impressive group of bright graduate students, some also refugees, like Kurt Bloch, and many drawn from New York's City College and destined to become leading figures in biochemistry–David Shemin, Seymour Cohen, and Boris Magasanik to name some. It was, however, to Michael Heidelberger's laboratory in the Department of Medicine that I was particularly drawn. Heidelberger, proud of his training as an organic chemist, had, together with Oswald Avery, discovered that the major antigens of pathogenic pneumococci were polysaccharides. One of the consequences of this momentous discovery was that antibodies were finally and unambiguously shown to be proteins (in immune precipitates made with purified polysaccharide antigen). It also led to quantitative precipitation assays to measure antibody concentrations in serum, in weight units, in contrast to the prevailing methods of determining titers by serial dilution. Titers could be determined rapidly and easily and, in the hands of skillful analysts and with appropriate sera, could yield unambiguous results. This was the case, for example, in all of Landsteiner's elegant work in determining the structural basis for antibody specificity. But titer determinations could also be subjective and misleading, as was soon demonstrated in a study I carried out with Manfred Mayer, a graduate student in the Heidelberger lab at the time and later a leading figure in the study of complement proteins.

A series of papers had appeared showing that when adrenal cortical steroids were injected along with antigen into rats, the resulting antibody titers substantially exceeded those found after injecting the antigen alone, indicating that these hormones, involved in general stress responses, enhanced antibody production. When we repeated the work, measuring serum antibodies by quantitative precipitin reactions, we could find no effect from the adrenal hormones. The results brought to an abrupt halt a line of investigation that was receiving wide attention because of its implications for enhancing human immune responses.

Following a return to Bellevue Hospital and a medical residency, I served during an extended vacation as a ship's surgeon on a boat carrying cargo down the west coast of South America and bananas back to New York. Though intermittently busy treating injuries resulting from loading and unloading of cargo and administering bismuth or arsenicals to treat crewmen with syphilis, I had much free time for reading in solitude on the uppermost hurricane deck. I had brought along the revised edition of Landsteiner's monograph on the "Specificity of Serological Reactions" and read most of it. Published at the time of Landsteiner's death in 1943, it was a superb summary of what was known about immunology at the time, and the final chapter, by Linus Pauling, was a wonderful description of noncovalent molecular interactions. Though it was largely addressed to Landsteiner's work on the reactions of antisera with small organic molecules (haptens), the view it provided of molecular complementarity as the basis for antigen recognition by antibodies doubtless helped cement my interest in the immune system, and particularly in the use of structurally well-defined small molecules as substitutes for more complex antigens, such as the red blood cells and bacteria then (and subsequently) commonly used as antigens to study immune responses. There was, however, no obvious way to pursue that interest, for opportunities to engage in research were then limited.

At the time, the academically inclined physicians I knew carried out research in a few hours taken away from busy practices, using all sorts of make-shift arrangements. But all of this was shortly to change as the federal government became committed to support research in universities, including medical schools. The change was due largely to World War II when, as described in Daniel Kevles' superb article (*Daedalus* 121:195–235 [1992]), "Physics won (the war) with microwave radar, proximity fuses, and solid-fuel rockets, and ... ended it with the atomic bomb." Before the war, biomedical research was almost entirely dependent on a few philanthropists and philanthropic institutions who distributed their largess to a small number of highly selected, elite institutions. NYU was definitely not one of them. After the war, however, federal funds became more widely distributed—not only to physics and chemistry, of obvious interest to the defense establishment, but to biomedical research as well. The National Institutes of Health (NIH) expanded and devoted a sizeable proportion of its funds to support research fellowships for "promising young biomedical scientists and for basic and applied biomedical research conducted in universities and medical schools."

As an early beneficiary of that commitment, I received a senior NIH fellowship in 1948. A forerunner of today's physician-scientist awards, it could not have come at a better time. I was then about to make the most important move in my life in marrying Natalie Aronson and starting to raise a family that would ultimately include five children. The fellowship facilitated this momentous step by providing a generous stipend ($3600/yr), allowing me to devote myself full-time to research for two years. My official mentor at NYU was William Tillett, chairman of the Department of Medicine who, fortunately, seemed not interested in what I did or where I did it. As I was drawn to chemistry, I spent time in the Department of Biochemistry, and the chairman, R. Keith Canaan, a courtly Englishmen who enjoyed a considerable reputation for his early work on the acid-base titration of ovalbumin, graciously provided me with bench space in his lab. There I was completely free to pursue whatever interests I had. That freedom, which I then took for granted, was an extraordinary opportunity, now only available in rare special postdoctoral fellowships, like those at the Whitehead Institute at Massachusetts Institute of Technology: the several remarkably accomplished young scientists who have emerged from that program in recent years testify to the power of early independence for young scientists.

In Canaan's lab I was bent on using low mol. wt. molecules—haptens—to study immune reactions of medical interest. I visualized taking advantage of them to study hypersensitivity reactions and so turned to sulfonamides. These antibacterial drugs were revolutionizing the treatment of many infectious diseases, but one major drawback was that they occasionally caused severe allergic reactions. The attractiveness of using such well-defined molecules was enhanced by the controversies surrounding studies of hypersensitivity reactions to conventional protein antigens, such as diphtheria toxin. Later, at meetings of the American Association of Immunologists, Elvin Kabat's criticisms of work with purified bacterial toxins led to unwary victims of his formidable verbal skills being referred to as having been "kabatized," rather than baptized, by the their first public encounter with a scientific controversy. With low mol. wt. antigenic surrogates, however, critical reactants could be unambiguously identified by specific inhibition with other, structurally related simple molecules, e.g., using hapten inhibition in vivo, paralleling its established use in vitro, to define the specificity of hypersensitivity reactions.

BINDING OF HAPTENS TO ANTIBODIES

The plan to study immune reactions was put in motion but then set aside temporarily in favor of another that grew from the work being done by Fred Karush, another postdoctoral fellow with whom I happened to share bench space in Canaan's lab. Karush was using equilibrium dialysis to study the reversible binding of dodecyl sulfates to bovine serum albumin while I was learning how to generate and purify antibodies to diazotized small molecules. We decided to join forces, using equilibrium dialysis to study the binding of small dialyzable molecules (haptens)

to purified antibodies. Fred had received a PhD in physical chemistry from the University of Chicago, spent a year in physics of MIT, and then gone to work as a paint chemist at DuPont to support his family. But, eager to return to academia and to study proteins, he obtained a three-year National Research Council fellowship, starting in Canaan's lab at NYU. By sheer luck, our disparate interests and backgrounds were joined in a collaborative study that marked a turning point for each of us. From Fred I learned to appreciate the elegance of physical chemistry; he may have learned a bit of biology and medicine from me; and we both came away from the collaboration with a lifelong friendship that involved as well our respective families.

To study the binding of an azodye to antibody, we turned to azobenzenearsonate (called R) because it was being extensively used by Pauling, Pressman, and Campbell to analyze antibody reactions with polyvalent azodyes. Anti-R antibodies were purified from rabbit antisera (raised against Razo-protein conjugates) with the aid of insoluble adsorbants made by coupling the Razo group to red blood cell stroma, in a primitive form of what was later called affinity chromatography. With conventional dialysis membranes, which were permeable to small azo dyes but not to antibodies, it was clear from the first experiment that when one started with a colorless antibody solution on one side of the membrane and a solution of a red Razo-phenol dye on the other side, the red dye became obviously concentrated on the side containing the antibody. More than the exciting visual effect, by varying the initial dye concentration and thereby the amount of hapten bound, it was a straightforward matter to determine both the equilibrium binding constant of the binding reaction (the antibody's affinity for the dye) and the number of binding sites per antibody molecule.

ANTIBODY VALENCE: The Number of Binding Sites Per Antibody Molecule

The number of binding sites was much debated at the time, in part because of its implications for how antibodies are generated. From the ability of some immune precipitates to bind additional antigen (proteins or polysaccharides), Heidelberger and Kendall inferred that antibodies had multiple binding sites for antigen. Focusing on precipitin reactions in which conventional high mol. wt. antigens were replaced by azodyes having multiple haptenic groups per molecule, Pauling considered the results in his lab to justify his preference for two sites per antibody molecule, the simplest form of multivalency. Heidelberger, who evidently preferred more than two sites (to fit the view of the immune precipitate as a 3-dimensional lattice, formed by linking antibodies and antigens in widely varying ratios), mentioned, with obvious satisfaction in one of his many reminiscences, having observing evidence for trivalent antibody in one of Pauling's lectures. Felix Haurowitz differed from both, however, and insisted that there was a single binding site per antibody molecule.

Why the insistence on univalence? Haurowitz, while living temporarily in Constantinople as a refugee from Hitler's Germany, had initially proposed, with Breinl, the antigen-template theory of antibody formation. In the theory's refined form advanced about 10 years later by Pauling, the antibody molecule was viewed as a single polypeptide chain that, as a newly synthesized nascent molecule, folded up around an antigen template, thereby acquiring two binding sites complementary in shape to antigenic sites (now called epitopes, after Jerne). Since antigens characteristically have many different epitopes per molecule—assume two for simplicity, A and B—some bivalent antibodies would have two anti-A sites and others two anti-B sites, but the majority would be bispecific, i.e. with one anti-A site and the other, on the same molecule, being anti-B (the ratio of their frequencies being 1:1:2, according to Pauling). Aware of this possibility, Haurowitz had searched for antibodies with two different specificities but could not find them. Therefore, being totally wed to the antigen-template theory (as was everyone else at the time), he maintained that antibodies had to be univalent.

When Karush and I published our evidence for two anti-R sites per antibody molecule (in the *J. Am. Chem. Soc.*, where papers on antibodies from the Pauling lab had been regularly appearing), the response was mostly gratifying. Pauling sent us a congratulatory letter, noting especially his pleasure at seeing the idea of bivalency confirmed. Haurowitz ignored the results and continued to maintain for years that antibodies are univalent.

BIVALENCE VS. BISPECIFICITY

We returned to the issue of antibody bispecificity shortly afterwards, analyzing antibodies produced in response to 2,4-dinitophenyl-bovine gamma globulin (DNP-BGG): The antisera contained abundant levels of anti-DNP antibodies and even more anti-BGG antibodies, but, as found previously by Haurowitz, there was no evidence for bispecific antibodies–e.g., there were no anti-BGG molecules that had anti-DNP activity. That antibodies are bivalent but not bispecific was disquieting, but few remarked about it. Kabat (not known for commenting favorably about other people's work) was one of the few. Yet, neither he nor I nor anyone else at the time was sufficiently motivated by this apparent disconnect between theory and observation to think seriously about alternatives to the antigen-template hypothesis. To those not active in immunology at the time it is probably impossible to imagine the powerful hold this hypothesis had for over 20 years, until the publication of Burnet's monograph on clonal selection and independent powerful evidence from Anfinsen and colleagues that a protein's conformation, including the shape of its binding sites, is dictated by its amino acid sequence.

Having experienced the excitement of laboratory research, I was not ready to leave it for the private practice of medicine, then the only obvious means for supporting a family. Karush had moved to a temporary position at the Sloan Kettering Institute in David Pressman's group, and I was then also offered a job in

Pressman's program, which involved the tissue localization in mice of radioiodinated antibodies raised against tissue antigens, a foreshadowing of modern efforts for the immunotherapy of cancer. I was not excited by the program and after 10 months accepted a position back at NYU in a newly formed Department of Industrial Medicine.

DELAYED-TYPE HYPERSENSITIVITY SKIN REACTIONS

The Department had received a gift from the Standard Oil Company of NJ to set up a lab to study diseases of significance for the company. I was not sure what they had in mind or why the position was offered to me, but it provided a total of $10,000/yr to start up a new lab, hire a technician, and pay me a salary that would have to be augmented by part-time private practice. I had no difficulty in deciding to focus on allergic skin reactions to dinitrobenzenes, to take advantage of both Landsteiner's early work on delayed type hypersensitivity (DTH) to these compounds and Fred Sanger's landmark use of 2,4-dinitrophenyl (DNP) amino acids to establish that proteins are linear polymers of amino acids joined in alpha peptide bonds, a concept that at the time was still being debated. As a postdoctoral fellow, I had heard Sanger's seminar a couple of years before, describing how he attached DNP to the N-terminal amino acid of an insulin chain and to its various proteolytic fragments; then, after hydrolysis, he identified the released DNP-amino acids by silica gel chromatography. Intending to apply this approach to purified antibodies, I had synthesized 2,4-dinitrofluorobenzene (DNFB was used to prepare DNP-amino acids and peptides but was not then available commercially). I abandoned the project when I become sensitized to DNFB, evidently by handling it carelessly, and developed severe contact dermatitis. Thus, when I started the study of DTH reactions to dinitrobenzenes a few years later, I had my own skin reactions to turn to for experimental purposes, as well as those of deliberately sensitized guinea pigs.

A large number of 2,4-dinitrobenzene derivatives, including DNFB, were tested on both DNFB-sensitized guinea pigs and on my own skin, using the simple patch tests commonly used by dermatologists. From hydrolysates of skin from guinea pigs painted with some dinitrobenzenes, DNP-amino acid could be identified using Sanger's chromatographic systems. The results confirmed Landsteiner's conclusion, drawn from the use of phenol at high pH as a model protein: 2,4-dinitrobenzenes having good "leaving" groups in the carbon-1 position reacted well with protein and elicited the delayed-type hypersensitivity skin reaction. This property divided the dinitrobenzenes distinctly into those that could or could not elicit these allergic reactions; with one exception, the results with the guinea pigs and my own skin were entirely concordant.

The exception, 2,4-dinitrobenzesulfonic acid (DNBSO3), was inactive on the sensitized guinea pigs but elicited responses on my skin. As the sulfonate derivative was water soluble, it seemed that the difference could have been due to the presence

of sweat ducts in human skin and their absence in guinea pig skin. (The idea was, incidentally, supported by applying a patch test on my skin at the edge of an old vaccination scar and seeing the typical DTH allergic skin reaction develop on the normal skin but not on the contiguous scar, presumably because scars lack sweat ducts.) The reaction on human skin indicated that DNBSO3 formed DNP-proteins in situ, and we showed that it was indeed a highly effective reagent for preparing water soluble DNP-proteins; it also had the added advantage over DNFB of reacting almost exclusively with lysine side chains.

WASHINGTON UNIVERSITY MEDICAL SCHOOL IN ST. LOUIS

The arrangement made initially with the Industrial Medicine Department was that I would spend half-time in research, allowing the other half for medical practice. Instead, I came to spend increasingly long days in the lab and to see patients only in the evening. This was fine for research, but unfair to patients, and even more unfair to our growing family. The publications, however, were not unnoticed, and one day in the lab I received a phone call from W. Barry Wood who wanted to know if I would be interested in a position in the Department of Medicine, of which he was chairman, at Washington University's medical school in St. Louis. He had persuaded the Rockefeller Foundation to endow a chair in Dermatology, within his department, arguing that research on skin disease, traditionally carried out within Departments of Dermatology, were languishing, unlike the vigorous research being carried out in many departments of internal medicine on diseases of the kidney, cardiovascular system, etc. I was thus offered an endowed chair as Professor of Medicine and head of the dermatology service of Barnes Hospital, the medical school's great teaching hospital, even though I had had no training in dermatology. With a generous salary and liberal lab space, it meant not only enhanced research opportunities but an end to exhausting moonlighting in private medical practice. Before I could accept the offer, Wood let me know that he was returning to Johns Hopkins, where he had previously spent many years, and that he would be succeeded as department chief by Carl Moore. Part of the offer's attraction was Woods great reputation as a charismatic leader in academic medicine. But Moore was also enormously respected for his intellect and character, and once I had met him any doubts I may have had vanished. I accepted the offer enthusiastically.

Moore proved to be a wonderful colleague and in time a close friend. Though Wood had moved to Johns Hopkins we became close collaborators when, with Benard Davis (Harvard), Harry Ginsberg (University of Pennsylvania), and Renato Dulbecco (Salk Institute), we wrote a new textbook of microbiology. I was responsible for the immunology chapters. Intended to differ from traditional textbooks, it sought to describe not only what was known at the time, but how we got to know it. The effort was initially exhilarating; the first edition came to be widely used; and the Immunology section was subsequently published as a series

of separate volumes. After an equally satisfactory second edition, the later ones suffered from my lack of first-hand experience with the emerging new information about cellular immunology, which had to be incorporated. The comments about textbooks in general in Thomas Kuhn's quotation at the beginning of this article could well apply to the final edition, appearing about 20 years after the first one. It was a relief to end the series.

When we first arrived in St. Louis renovations for my lab were still underway and would not be completed for about six months. Arthur Kornberg, head of Microbiology, generously offered me space in a lab where all of the department's graduate students worked, clustered together by design, rather than in their mentors' individual labs. The department was small and populated by a young and extraordinarily talented faculty and group of postdoctoral fellows, and a daily journal club at the communal lunch added to the general level of excitment about science.

QUENCHING ANTIBODY FLUORESCENCE

To analyze antigen recognition by antibodies in greater detail than had previously been possible, we wanted to measure differences in free energies of binding of various haptens to purified antibodies. When DNBSO3 was used to produce highly substituted, water soluble DNP-proteins as antigens, which were administered in complete Freund's adjuvant, the resulting rabbit antisera contained suprisingly abundant amounts of anti-DNP antibodies, often around 50 times more than we had previously obtained with different immunization procedures, and the antibody could be easily isolated in highly purified form. I told Sidney Velick about these wonderful antibodies, and he in turn told me about the wonderful procedure he was using to analyze the binding of pyridine nucleotides to a dehydrogenase enzyme: The procedure was based on the transfer of energy emitted by UV light-activated tryptophan residues of the protein to bound ligand. The resultant quenching of the tryptophan's fluorescence emission depended in large measure on spectral overlap between the ligand's absorption spectrum and the tryptophan's emission spectrum. It took some time for me grasp the power of that approach, and I suspect that Velick was at first skeptical about the ready availability and purity of the isolated antibodies. But once the spectral overlap between tryptophan fluorescence emission and the absorption spectrum of the principal DNP ligands was appreciated, Velick carried out the first fluorometric titration of anti-DNP antibodies with e-DNP-lysine. The quenching of the antibodies' fluorescence by the bound DNP-hapten was dramatic, and it was immediately evident that the antibody we were dealing with had exceptionally high affinity for the DNP ligand, about 10,000-fold higher than the affinity of the anti-DNP antibodies (and the anti-benzenearsonate antibodies) we had analyzed previously in New York. Those antibodies had been elicited by immunization procedures then in vogue, which relied on injections of large amounts of alum-precipitated DNP (or Razo-) proteins.

AFFINITY MATURATION

Because fluorescence-quenching titrations required small amounts of antibody and could be carried out rapidly, it was possible for Gregory Siskind, who later came as a visitor to the lab, to analyze many samples of antibody isolated at different times after injecting rabbits with various quantities of antigen. The results revealed clearly that over time, after small amounts of antigen were injected, there was a progressive increase in the antibodies' affinity for ε-DNP-lysine, the ideal surrogate for the principal epitope of the immunizing protein.

It had long been known that after immunization antisera increased in "avidity" over time, i.e., in the stability of the complexes they formed with the antigen. But with conventional protein antigens, having various epitopes per molecule, increasing stability of the antibody-antigen complexes formed with antisera could come about for a variety of reasons, such as increasing diversity of the recognized epitopes. This complexity had led some to recommend that the term avidity be abandoned altogether because it could not be clearly defined. Even in Jerne's classic study of antisera neutralization of diphtheria toxin, where increasing stability over time was evident, the binding was complicated, the number and variety of epitopes per molecule of antigen (toxin) were unknown, and the antitoxin molecules were also not univalent. With small ligands such as ε-DNP-lysine, however it was clear that the antibodies' intrinsic affinities were measured. That the antibodies appearing initially and those appearing later differed in intrinsic affinity for the same ligand thus provided unambiguous evidence that the antibody binding sites changes over time. The progressive changes, later termed affinity maturation by Siskind and Benacerraf, were subsequently seen to occur with antibodies made against various other haptenic groups. The changes, together with the later finding of somatic hypermutation of antibody genes by Milstein, Berek, Rajewsky and others, provided a coherent (though still gross) view of how antigen recognition, as reflected in affinity for epitopes, can serve as a potent driving force for immune responses.

When clonal selection was first advanced it rested, many of us thought, on pretty skimpy evidence. Several direct attempts to "falsify" it (in the sense used by Hans Popper)—by determining whether single antibody-forming cells could produce two (or more) distinctly different antibodies—had yielded contradictory results. Affinity maturation did not really distinguish between this and the antigen template theory, which, for a very short time, could be viewed as a competing paradigm. Clonal selection provided an obvious explanation for affinity maturation, with progressively diminishing levels of antigen after immunization leading to increasingly selective stimulation of cells ("clones") making the higher affinity antibody. However, the serum antibody changes could also be explained by a treadmill mechanism, in which the affinities of the antibodies synthesized over time did not really change—it may have been that only the average affinity of serum antibodies increased as the levels of free antigen available to selectively bind and

remove high affinity antibodies diminished over time. The treadmill possibility was ruled out when Lisa Steiner joined the lab and showed that the antigen-binding activity of antibodies synthesized by isolated lymph node cells, collected at various times after immunization, paralleled changes in the intrinsic affinity of serum antibodies. Her additional finding that antibodies synthesized promptly in a secondary (memory) response to the antigen had the same high affinity as those made many months after the primary response was initiated were especially telling: They could be easily explained by clonal selection but not by the template theory.

Although cellular immunologists embraced clonal selection without a second thought, some immunochemists fought a rearguard action against it, finding the supporting evidence too skimpy. One of the battlegrounds was the heterogeneity of serum antibodies: Though an "average" intrinsic affinity could be assigned to purified anti-hapten antibodies, innumerable experiments had shown that virtually all preparations behaved as though they were mixtures of molecules having different affinities for a common ligand. According to clonal selection, the mixtures were obviously the polyclonal products of diverse antibody-producing clones responding to the same antigen. But to many immunochemists this heterogeneity was seen to reflect sloppiness in the folding of nascent antibody molecules (around an antigen-template), or even as an artefact, the result of inhomogeneity in the hapten-protein conjugates used to elict antibody production (i.e., with haptenic groups substituted in diverse amino acid residues of the carrier protein). We had found earlier that, even with a homogeneous DNP-protein as antigen [having a single DNP group attached to a specified residue (lysine 41) in bovine pancreatic ribonuclease], the elicited anti-DNP antibodies were just as heterogeneous in affinity for DNP-lysine as were the antibodies raised against conventional heterogeneous DNP-proteins antigens. Our results were published only in a Harvey Lecture, and we naively assumed they would settle the matter. But, as they were not in a peer-reviewed journal, they may have been overlooked. In any case, some skilled immunochemists subsequently wasted much effort ingeniously constructing homogeneous antigens.

MYELOMA PROTEINS AS HOMOGENEOUS (MONOCLONAL) ANTIBODIES

If the polyclonal explanation were correct, the antibody molecules produced by a single clone should bind the antigen with uniform affinity. The only candidates available at the time for such a test were myeloma proteins. Secreted by myeloma (plasma cell) tumors, they had the same basic heavy- and light-chain structure as antibodies, but even so, many, even Rod Porter, the astute discoverer of the multichain structure of immunoglobulins, regarded them as abnormal "paraproteins" because they were made by abnormal (cancer) cells. Given the enormous number of different antigens and the prevailing idea that an antibody recognizes a single

epitope, or a few similar ones, the chances of finding a myeloma protein that specifically bound any particular epitope were expected to be extremely small. We nevertheless undertook the search because an attractive means for rapidly screening serum samples from individuals carrying myeloma tumors could be visualized.

DNP amino acids undergo a prominent "red" spectral shift when bound to anti-DNP antibodies, probably because of so-called charge-transfer complexes formed by the bound ligand with a tryptophan residue in or very close to the antibody binding site. The shift appeared to be a distinctive marker for anti-DNP antibody binding sites in general, since we had consistently seen it with these antibodies from diverse sources (rabbits, guinea pigs, chickens, etc). When, however, a DNP amino acid bound to serum albumin, the abundant serum protein that binds (weakly) a great many different ligands, the absorption spectrum shifted in the opposite direction (to the "blue"). Thus, it seemed that simple spectrophotometric readings of myeloma serum samples to which DNP-lysine had been added would quickly reveal whether this ligand was bound to a myeloma protein that behaved like an antibody.

The opportunity to test this screening procedure turned up unexpectedly when Arthur Kornberg and the entire faculty in the Department of Microbiology moved from Washington University to Stanford. I was offered the chairmanship of the Washington Department with the tough mandate to rebuild it, but I welcomed the chance to move from a clinical to a preclinical department. One of the unexpected rewards of making the move was that I inherited a number of instruments that the former department members had chosen not to take with them, including an elegant double-beam Cary spectrophotometer. With the aid of that instrument and specially constructed cuvettes, it was possible to test a myeloma serum in a couple of minutes. The screening required a large number of serum samples, of course, and fortunately these were generously made available by Kurt Osterland, who had for years been collecting them from patients with myeloma tumors. When the screening got underway, we anticipated, as noted above, that we would be lucky to find one out of perhaps a thousand with the desired properties. Instead, one turned up within the first twenty tested. The active serum, called BRY (after the patient's family name) came from a patient who had left the hospital and was gone without a trace, and only three ml of her serum was available in Osterland's collection. That was sufficient to isolate enough of the myeloma protein (an IgG1 molecule) to show that it had two binding sites per molecule, one in each Fab fragment, and that DNP lysine bound to it in the same way as to conventional anti-DNP antibodies. It differed however in one important respect: The binding affinity was clearly homogeneous, unlike the heterogeneity that characterized all of the innumerable samples of anti-DNP antibodies isolated from immunized animals. The results supported other mounting evidence for the "one cell-one antibody" rule, the keystone of the clonal selection hypothesis.

AN EMBARRASSING ERROR

When the results with the human myeloma protein were presented at the 1967 Cold Spring Harbor Symposium (the first one devoted to immunology), Mike Potter offered to provide our screening program with serum from mice, in his large collection, carrying diverse myeloma tumors. Out of the first approximately 100 sera tested, two anti-DNP myeloma proteins were identified. The first one, MOPC-315, bound e-DNP-lysine strongly, and as the tumor was readily transplantable into normal BALB/c mice, large amounts of this monoclonal antibody could be expected and indeed were eventually produced. The paper describing its properties carried a triumphal note, but it was disturbing to realize shortly after publication that it had a couple of significant errors. I describe these in some detail below because they illustrate the self-correcting character that is inherent in the scientific enterprise. This aspect of science seems at times to be utterly incomprehensible to journalists, politicians, and the public at large—as I was to find out painfully many years later when enmeshed in the notorious case of alleged scientific fraud involving David Baltimore and Theresa Imanishi-Kari.

Based on peptide fingerprints, MOPC-315 was reported to have a kappa light chain. The error came about because Potter, a coauthor, had previously prepared fingerprints of several lambda light chains and seen that they were all were similar to each other but were distinctly unlike MOPC-315's light chain; hence the latter was considered to be a kappa chain, the only other light chain type known at the time. Ultimately this protein's light chain proved to be the first example of an uncommon lambda chain type, designated lambda-2. The other error was mine and less forgivable. To determine MOPC-315's affinity for DNP-lysine we had used especially small plastic chambers designed to carry out equilibrium dialysis with extremely small (50 μl) volumes and fabricated in our department's machine shop. Using the new chambers we found that the amount of radiolabeled ligand bound at saturation corresponded consistently to about 1.2 moles ligand per mole myeloma protein (antibody). As the number of sites had to be an integral number and was definitely not two, we concluded that it was one, i.e., that this ostensibly monoclonal antibody was univalent! The result was especially disconcerting because of the role Karush and I had played in establishing that antibodies are bivalent, which by then (about 15 years later) was universally accepted. But to some skeptics the apparent univalency confirmed their suspicion that myeloma proteins were indeed "paraproteins" and differed from conventional antibodies. That there really were two binding sites per molecule of myeloma protein, however, and not one as reported, became clear some time later when higher concentrations of MOPC-315 and of another anti-DNP myeloma protein had to be used in the small plastic chambers (to measure the equilibrium binding of weakly bound ligands), and the new results clearly pointed to two sites per molecule for each of these proteins. It then became apparent that some protein (with its bound ligand) was lost by adsorption to the chambers' plastic walls during the equilibration period. Though small in amount, the proportion of adsorbed protein was significant when low

concentrations of protein were introduced (as in the initial report) but negligible when high protein concentrations were used. To have been unaware that proteins bind to many plastic surfaces was particularly embarassing because "solid phase" assays for antibodies and antigens were being introduced at about that time and their effectiveness was known to derive from the firm binding of trace amounts of proteins to plastic surfaces.

What was done about the errors, once they were discovered? They were not publicized with great fanfare as errata or by letters of correction to the journals, as was later indignantly demanded by critics of the senior authors in the Baltimore case. Instead, the corrections were simply incorporated into later publications, along with other findings, as part of the normal self-correcting process.

IDIOTYPES OF MYELOMA PROTEINS AS TUMOR-SPECIFIC ANTIGENS

Since each myeloma protein was expected to have a unique antigen-binding site or idiotype, the question arose as to whether MOPC-315, which had its origin in a BALB/c mouse, could stimulate the other mice of this genetically uniform strain to produce antibodies to protein 315's idiotype. At the time Stitaya Sirishina was traveling in the United States on a Rockefeller Foundation fellowship with a view toward reorganizing the Department of Microbiology at the Mahidol University in Thailand. He became interested in the idiotype question and turned what was to be a brief visit into a prolonged stay, during which he showed that mice injected with anti-DNP myeloma proteins produced antibodies that were evidently specifically "anti-idiotypes," since their reactivity with the corresponding myeloma protein was blocked by DNP-compounds.

A myeloma protein's idiotype corresponds literally to a tumor-specific antigen, since it is uniquely a product of the tumor that produces it. But the protein is copiously secreted by the tumor cells, and it was not clear that enough of it was present on the surface of tumor cells to result in their destruction by the anti-idiotypic antibodies. Despite the uncertainty, Dick Lynch, then a postdoc in the lab, injected MOPC-315 cells into mice that had been immunized with purified protein 315. In nearly all mice, tumors failed to grow out. And in the few animals in which the tumors eventually appeared, they proved to be idiotype negative because they had ceased to produce the myeloma protein's heavy chain. Similar results were obtained with another hapten-specific myeloma protein, MOPC-460 (but not to a third one, of unknown specificity). Kristian Hannestad, who came as a sabbatical visitor, studied the problem further; on returning to Tromso, Norway, he and his colleagues continued to analyze the rejection mechanism. It is far more complex than was originally visualized: They have shown that T cells recognize peptides from a segment of light chain that contributes to the myeloma protein's ligand-binding site and that these T cells seem to be responsible for tumor cell destruction. The anti-idiotype approach was later extended by others to humans with B cell lymphomas, evidently with some occasional benefits.

THE FREQUENCY OF LIGAND-BINDING MYELOMA PROTEINS

The finding of several myeloma proteins with considerable affinity for nitrophenyl groups among the small number of myeloma sera screened was (and is) not expected because of the general view that (i) each individual produces an enormous number of different antibodies, and (ii) the reactivity of each antibody appears to be highly restricted to one antigen (and a few similar ones). To test the alternative possibility—that an antibody can bind more promiscuously to a variety of disparate epitopes—we chose, more or less at random, a large number of organic molecules (the aim was 57, the number appearing in a popular advertisement for the Heinz food company) to determine if any of them could inhibit the binding of a radiolabeled DNP ligand to MOPC-315. Several competitors were found by Maria Michaelides, the most active being menadione or vitamine K3 (2-methyl-1,4-naphthaquinone). It turned out that this ligand was also bound by several conventionally produced polyclonal antibodies to various dinitrophenyl- and trinitrophenyl-protein antigens, indicating a vague match in structure, corresponding to what was later labeled molecular mimicry by Oldstone. Several others examples of such "strange" cross reactions by antibodies, usually encountered serendipitously, have been noted. But with the advent of Milstein's and Kohler's powerful procedure for generating monoclonal antibodies, the extent to which individual myeloma proteins and conventional antibodies react with many disparate epitopes ceased to be of interest. Whether individual antibody-producing cells, B cells, can react with diverse antigens is an issue we revisit at the end of this chapter.

One of the unexpected benefits of studying hapten-specific myeloma proteins emerged from the efforts of David Givol and colleagues at the Weizmann Institute. While analyzing a pepsin digest of MOPC-315 with its bound ligand (a yellow DNP amino acid), they found an unusually small yellow fragment, its color indicating that it retained the ligand. The fragment proved to consist only of noncovalently associated variable domains of the light and heavy chains ($V_L + V_H$). Called Fv, it accounted for all the hapten-binding activity of the intact protein. Single chain Fv recombinant proteins, made with V_L and V_H linked covalently by a flexible, short polypeptide chain, were subsequently prepared by many others from various monoclonal antibodies for use as potential therapeutic agents (e.g., linked to a toxic protein in so-called immunotoxins).

A MOVE TO MIT AND T CELLS

I joined the Center for Cancer Research at the Massachusetts Institute of Technology (MIT) when it opened in 1973. Salvator Luria, its founding director and guiding spirit, had written to me about a role for immunology in the Center and

our correspondence led to an offer to join it. This came at a time when I was having to spend much time and energy on medical school administrative affairs and was eager to become again more fully immersed in the laboratory. The offer was attractive moreover because MIT was close to Woods Hole (on Cape Cod), where our family had enjoyed summer vacations for many years. And so, after having experienced academic life only in medical schools for over 30 years, and having particularly enjoyed it at Washington University, I found myself transplanted into a totally different academic scene. At MIT science was everywhere. The Cancer Center was an integral part of the Biology Department, and both were populated by a remarkable group of talented and energetic faculty. And in the Harvard-MIT Program for Health Sciences and Technology, with which I was associated for a time, interesting interactions with colleagues in physics and chemistry and the engineering sciences were commonplace. MIT seemed a form of scientific heaven. And, reinforced by the opening of the closely affiliated Whitehead Institute a few years ago, it still does as I write this 27 years later.

At MIT our interests in antigen recognition gradually shifted from antibodies to T cells. When the distinction between B and T cells first became apparent (in the early 1960s), it greatly excited cellular immunologists. The excitement was not shared by most chemically-minded immunologists, who initially were disdainful of what they perceived as a lack of rigor. A prominent immunochemist friend once asked me gleefully if I was aware that B and T were the first and last letters of b ... t, the ubiquitous barnyard substance. It was clear, nevertheless, that T cells could transfer delayed type hypersensitivity (DTH) skin reactions from sensitized to normal recipient animals, indicating that T cells expressed antigen-specific receptors (T cell receptors or TCR) and that T cell–mediated reactions could be as specific as those due to antibodies. For example, I had been sensitized by 2,4-dinitrofluorobenzene, as noted above, and I responded to patch tests on my own skin to various nitrobenzenes only if they formed 2,4-dinitrophenyl derivatives of skin proteins in situ (in guinea pigs), but not if the derivatives were 2, 6-dinitrophenyl or 2,4,6-trinitrophenyl.

T CELL CLONES

When procedures became available for generating mouse T cell clones and growing them in culture with retention of normal function for prolonged periods, the temptation to study antigen recognition by these cells became irresistible. We concentrated on CD8 T cells primarily because they could kill cancer cells rapidly in test tube assays. For a time each new postdoctoral fellow coming to the lab was asked to generate T cell clones. Many were produced, and we concentrated on those clones that grew readily and could be maintained in culture for years.

One of the better clones was called 2C. Generated initially by Mischa Sitkovsky, then a recent immigrant from the Soviet Union, it was nurtured and developed by

David Kranz, who also generated a monoclonal antibody that reacted exclusively with the TCR on 2C cells. These cells served as the basis for a collaborative effort with Susumu Tonegawa that resulted in cloning the genes for the α and β subunits of the clone's TCR, shortly after genes for TCR β subunits had been cloned independently by Mark Davis and Tak Mak. The initial paper describing the complete primary structure of the 2C TCR was written rapidly with Tonegawa et al and appeared in print (in *Nature*) around a month after the manuscript was started. Carrying a triumphal note as the first complete sequence of both the α and β subunits of a TCR (the 2C TCR), the paper could serve as a model for how speed, borne of unrestrained competiveness, can lead to major errors: What the paper called the α gene turned out to code for a subunit (γ) of what was soon realized to be part of the $\gamma\delta$ TCR on a special small T cell subset, called $\gamma\delta$ T cells, having quite different functions. The β gene described was also not the correct one for the 2C TCR. Eventually, however, the correct α and β genes for the TCR on the 2C clone were established with Kranz's help when Sha, in Dennis Loh's lab at Washington University Medical School, expressed them as transgenes in mice.

ANTIGEN RECOGNITION BY A T CELL

What does the 2C TCR recognize? Following the Zinkernagle and Doherty finding that MHC proteins "restrict" antigen recognition by T cells, and the findings by Unanue and Townsend and others that short peptides arising as proteolytic fragments from proteins, intracellular or other, associate with the MHC, it was evident that TCR generally recognize peptide-MHC complexes in which the MHC component restricts recognition of the peptide. When the 2C clone was first derived, it was readily apparent that its TCR recognized Ld, a non-self (or allogeneic) class I MHC protein present on the cells used to immunize mice that lacked this particular protein. But identification of the peptide associated with Ld required the heroic efforts of Keiko Udaka and Ted Tsomides, who systematically analyzed the myriad of peptides in mouse spleen extracts, separating them chromatographically (by HPLC) and painstakingly identifying active fractions by the cytolytic responses of 2C cells. From spleens of around 1000 mice they ultimately purified two peptides. Their overlapping amino acid sequences led to the identity of their source, which turned out to be a mitochondrial "housekeeping" protein (α keto-glutarate dehydrogenase), expressed in all cells.

As with virtually all T cells, the 2C TCR also has to recognize an indigenous (self) MHC protein in order to complete its maturation in the thymus. For T cells expressing the 2C TCR, this self-MHC was shown to be Kb in breeding experiments with 2C transgenic mice. This TCR can thus recognize peptide-Kb as well as peptide-Ld complexes, including those in which the same peptide (from α keto-glutarate dehydrogenase) is associated with Ld and Kb. Some years later, Tallquist and Pease identified another peptide, from a different mitochondrial protein, that is recognized by the 2C TCR in association with Kb.

DEGENERACY IN ANTIGEN RECOGNITION BY 2C T CELLS

How many other peptide-MHC complexes do 2C cells recognize? The recognition of complexes by a TCR is easily evaluated from the responses of CD8 T cells to target cells that express a restricting MHC protein and are incubated with various synthetic peptides. By binding to the MHC, the peptides form peptide-MHC complexes on the target cells. If the complexes are recognized by cytolytic T cells the target cells are destroyed. Simple cytolytic assays of this kind have determined that the 2C TCR can recognize a great many different peptide-MHC complexes involving at least 12 peptides (some with overlapping sequences) in association with two restricting MHC proteins (Kb and Ld). They also respond to another MHC protein, from a third MHC locus (H-2^r), probably in association with still other peptides. It also appears from the maturation of these cells in mice lacking classical class I MHC proteins that they can recognize a class I MHC of the so-called nonclassical type, which possibly binds some glycolipids instead of short peptides in its binding groove. The ability to recognize and respond to so many different structures warrants a distinct term and degeneracy seems appropriate. Degeneracy has been seen previously to various extents, as in reactions attributed to molecular mimicry by Oldstone and in Wucherpfenig's and Strominger's elegant study with T cells that react with a peptide from myelin basic protein as well as many viral peptides. There is no good reason to doubt that many other TCR would exhibit similar degrees of degeneracy if examined thoroughly.

T CELLS CAN DISCRIMINATE SHARPLY BETWEEN SIMILAR STRUCTURES

Although 2C T cells exhibit much degeneracy, they can also display exquisite specificity in discriminating between very similar epitopes. For example, these T cells lyse target cells presenting a particular peptide-MHC complex, call it A, but not the same target cells that present the same number of a slightly different complex, call it A′, where A and A′ bind equally well to the MHC molecule and where the difference between A and A′ is a single O atom resulting from a phenylalanine-tyrosine substitution in the peptide. This sharp selectivity by the TCR matches the high degree of specificity seen in the most discriminating reactions of antibodies or enzymes.

SPECIFICITY VERSUS DEGENERACY

How can a single TCR display exquisite specificity in some reactions and such extensive degeneracy in others? An explanation for specificity is not hard to discern. Specificity, the capacity to discriminate between two similar structures, depends not only on the difference in strength (call it affinity) of the two reactions but on how they are detected. Since all detection systems have a threshold, below

which reactions cannot be detected, a pair of ligands whose strengths of reaction straddle the threshold can be sharply distinguished. This, in fact, is the situation with the above cited example of the A and A′ epitopes that differ only by a phenylalanine-tyrosine substitution (i.e., by 1 O atom): Epitope (A), which elicits target cell lysis, has the lowest affinity measureable for a TCR-epitope reaction (about 3×10^3 M^{-1}), which is probably at the threshold, and for epitope A′ the affinity is probably just below the threshold.

Degeneracy is more intriguing because of its implications for the hypothesis that has served as the guiding paradigm for immunology over the past 40 years. The degenerate and specific reactions we are referring to here have been detected primarily by cytolytic assays. The extent of target cell destruction in these assays depends not only upon their displaying a peptide-MHC complex (epitope) that is recognized by the TCR, but upon the number of copies of epitope per target cell ("epitope density"). It also depends upon the affinity of the TCR for the epitope (i.e., on the equilibrium constant for the TCR-epitope reaction). And from studies carried out with Yuri Sykulev, Richard Cohen, and Ted Tsomides, it appears that high epitope densities are required for low-affinity reactions while low epitope densities suffice for high-affinity reactions, where affinity refers explicitly to the equilibrium constant for the binding of a peptide-MHC complex (epitope) to the TCR. This inverse relationship calls to mind the law of mass action, the fundamental rule for reversible chemical reactions in solution, although the application of this law to reactions between the TCR and peptide-MHC complexes, each embedded in a cell surface membrane, requires assumptions that are doubtless overly simplistic.

ONE CELL—MANY SPECIFICITIES

The central principle of the clonal selection hypothesis, the "1 cell–1 antibody" rule, has been repeatedly confirmed. It has been extended, almost subliminally, to mean "1 cell–1 specificity" because an antibody molecule characteristically reacts, as ordinarily measured, with a single antigen and a few structurally similar ones. For T cells, the corresponding 1 cell–1 TCR rule is also correct (although some T cells can have two TCR owing to the absence of allelic exclusion for the α subunit of $\alpha\beta$ TCR). However, the responsiveness of T cells to diverse epitopes indicates that the "1 cell–1 specificity" rule for these cells is inappropriate. A more reasonable slogan would be "1 cell–many specificities," with the magnitude of "many" still to be determined.

WHY T CELLS EXHIBIT MORE DEGENERACY THAN ANTIBODIES

Given the great structural similarity between antibodies and TCR, why should T cells, via their TCR, display so much more degeneracy than antibodies? The likely answer (aside from the possibility that TCR binding sites are more flexible

and conformationally adaptable to ligands than are antibody binding sites) is that very few of the many TCR molecules on a T cell's surface (perhaps only one or two out of around 100,000) have to be initially engaged in a stable TCR-epitope complex to trigger a T cell response. This great sensitivity stems from powerful amplification effects of signal transduction. Moreover, the capacity to recognize—i.e., to respond to—so many different epitopes reflects the importance of epitope density on antigen presenting cells. At a sufficiently high epitope density, even a very weakly recognized epitope can generate enough stable complexes with the TCR to trigger a T cell response.

ARE B CELLS SIMILARLY DEGENERATE?

The foregoing view may also apply to B cells. An indication that they can exhibit degeneracy can be seen in the levels of serum proteins following injections of antigens in conventional immunization procedures. It has been observed that antisera have elevated levels of total globulin that greatly exceed the levels of the antibodies that react specifically, in conventional assays, with the administered antigen. It may be that Jerne, in proposing the anti-idiotype network, was influenced by this difference. A more likely explanation than an anti-idiotypic network is that B cell responses in vivo are substantially degenerate, as are the T cell responses considered above. Thus, administration of an antigen (call it X) results in stimulating not only those B cells whose secreted Igs function as recognizable anti-X antibodies, but probably also many other B cells, whose secreted Ig molecules have too low an affinity for X to qualify as anti-X antibodies. When normal B cell clones become available, it may be feasible to test this possibility.

We have been told intermittently over the past thirty years that the end of the quest to understand the immune system is in sight. A distinguished immunologist was recently quoted in the *New York Times* as having said that we are within "a whisper" of understanding the immune system. The current level of understanding is indeed truly impressive. Monumental is not too much of an exaggeration, compared to the level of understanding in evidence at the first meeting of the American Immunologists I attended in 1949: There were about 50 or 60 people in attendance out of the total membership at that time of about 250. Yet, current efforts to engage the immune system to protect against cancers or the AIDS pandemic or malaria or tuberculosis, or to suppress the often devastating effects of autoimmunity, are still glaringly ineffectual. They stand as a stark reminder of how much more remains to be understood and effectively applied.

References were omitted here to conserve space. Anyone interested in the publications referred to in this article can find them at the following web site: http://web.mit.edu/biology/www/Ar/eisen.html (click on References).

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