The Meandering 45-Year Odyssey of a Clinical Immunologist

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Abstract

My work on basic and clinical immunology has focused on the regulation of the human immune response and how its disregulation can lead to immunodeficiency, autoimmune and malignant disorders. We translated insights gained from this fundamental research to the development of new approaches for the treatment of patients. Early in my career I focused the definition of novel pathogenic mechanisms underlying hypogammaglobulinemia including endogenous hypercatabolism of immunoglobulins as well as on the short survival of immunoglobulin molecules associated with protein-losing gastroenteropathy. Furthermore, the initial demonstration of active suppression by human suppressor T cells changed thinking about the pathogenesis of certain immunodeficiency disorders, including HTLV-I-- associated CD4+ and CD25+ expressing adult T-cell leukemia.

Recently my work has focused on the cytokines IL-2 and IL-15. As part of the study of our IL-2 receptor directed therapy of HTLV-I—associated adult T-cell leukemia, we codiscovered the cytokine IL-15 that stimulates T-cell proliferation and NK-cell development. For its action in NK and T-cells IL-15 utilizes a multisubunit receptor that includes a specific IL-15 alpha receptor element as well as two receptor subunits,` IL-2R∃ and the common gamma ((c) chain that is shared with IL-2. As a consequence of their use of common receptor subunits IL-2 and IL-15 share some functions especially in innate, NK, mediated immunity. However in adaptive immune responses, the two cytokines have distinct and competitive functions. IL-2 is a critical factor required for activation induced cell death (AICD) that leads to the suicide of self --reactive lymphyocytes. Thus, IL-2 favors peripheral tolerance to self antigens. In contrast, IL-15

inhibits AICD and facilitates the development and persistence of memory phenotype CD8 T-cells; that is IL-15 favors the persistence of lymphocytes involved in the memory and effector responses to invading pathogens. These actions of IL-15 carry with them the risk, that is realized, that disorders of IL-15 expression could lead to the development of inflammatory autoimmune diseases. These studies of the IL-2 and IL-15 system have culminated in the recognition that the IL-2 and IL-15 cytokine receptors represent extraordinarily useful therapeutic targets. The scientific basis for this strategy is that most normal resting cells do not express the alpha subunit of IL-2 receptor whereas this subunit is expressed by malignant T-cells, select T-cells involved in autoimmune diseases as well as in allograft rejection. To exploit this difference we have introduced diverse IL-2R directed agents including the unmodified murine anti-Tac monoclonal antibody (anti-CD25), this antibody armed with $\forall$ and $\exists$ emitting radionuclides, as well as a humanized form of anti-Tac (daclizumab) now approved by the FDA for the prevention of renal allograft rejection. The development of new forms of anti-IL-2R and IL-15R directed therapy is continuing to provide novel perspectives for the treatment of certain neoplastic diseases, autoimmune disorders, and the prevention of allograft rejection.

Introduction to Research

It was 3 A.M. on a cold February day in 1954, when Sherman Weissman, my friend and classmate from the University of Chicago and Harvard Medical School opened the curtains around my hospital bed on a ward at the Peter Brent Brigham Hospital in Boston, where I was a patient with a minor condition. He handed me my huge blue winter coat that I had used on the Chicago Midway and we silently sneaked out of the hospital to a small laboratory in the Harvard Dental School. We had received a $50 grant and permission to use a niche in a lab to try to extend the observations made by Allan Erslev who had just reported the discovery of a novel erythropoiesis stimulating factor, he called erythropoietin. Our investigations involved phenylhydrazine-induced anemia in rabbits. While I had been in the hospital, a friend substituting for me had held one of the rabbits inadequately leading to its death. Sherm and I decided we could not afford to lose another rabbit so each night I would sneak out of the hospital to continue the studies until morning light. This represented the dawn of my research career. The 1950's were also the time of awakening of the research field concerning non-endocrine growth factors, as well as that of modern immunology in general. During this era Byron Waksman, a professor in the medical school, was allotted only one hour to summarize all that was important and then known in immunology. Our brief laboratory experience was a critical one for both Sherm and me. When for the 45th Harvard Medical School (HMS) reunion report, he was asked to comment on a teacher, incident or situation at HMS that had a telling influence on his life, he chose as pivotal to the initiation of his research career, "thanks to someone unknown who left supplies out so Tom Waldmann and I could sneak into the lab at night to try to reproduce early

erythropoietin experiments". In parallel, this experience exposed me to the excitement of biomedical research and also provided me with an entrée into the NIH in the era of the doctor's draft, when only a small proportion of applicants were being accepted.

The Beginnings

I was born in New York City on September 21, 1930, the only child of Elizabeth Sipös and Charles Waldmann. My father and mother had emigrated from Nitra, Czechoslovakia in 1920 and from Budapest, Hungary in 1927, respectively. My mother was a teacher with her own elementary school in Budapest. Later in the United States she founded and ran her own nursery school. My father was an engineer educated at the Royal University for Engineers in Budapest, graduating with degrees in civil, mechanical and electrical engineering. His career in the United States and my early life were dominated by the moods of the Great Depression and by World War II. Although he had been the chief engineer of the Comstock Industrial Construction Company with the crash in the stock market and the onset of the depression the company declared bankruptcy and my father was out of work in the depths of the depression. In 1936 he joined the U. S. Civil Service during the New Deal era as an engineer in housing development working in the National Housing Authority of the Resettlement Administration in Washington, D.C. where he was involved in the construction of such communities as The Greenbelt Towns in Maryland.

After the war, in 1946 my father became chief engineer and member of the board of directors of American Community Builders, a company involved in the generation from scratch of Park Forest, Illinois, a community of over 30,000 individuals that has been the subject of many sociological studies including "The Organization Man". From this home

base I attended the University of Chicago during the Robert Maynard Hutchin's era. It was a magnificent, intellectually challenging experience that forced all of the students of the college to become de facto philosophy majors who concentrated on the "great books" and who were challenged to address questions concerning fundamental values and broad life issues in small, very interactive classes.

One of my interests that has persisted from an early age through my initial decades at the NIH.was photography. At age five I was given a 15 cent camera. My father impressed with some of my results, trotted one of my first photographs to the local camera store where in exchange for permission to put it in the store window I was given a new camera worth a dollar and a quarter. This mini-triumph started a very pleasurable avocation. Through the years there have been many interconnections between my photographic and scientific efforts. In each arena I have tried to seek simplicity for impact and to approach challenges in maverick ways by thinking and seeking odd interconnections between diverse elements, a process that has proved pivotal in expressing originality in both photography and research. As noted above, in my 4 years at Harvard Medical School I not only learned to appreciate clinical medicine but was also introduced, albeit minimally, to research on growth factors.

In 1955-56 I was medical intern at the Massachusetts General Hospital where as the most pivotal event, I met Katharine Spreng, my medical resident. On March 29, 1958 we were married. The subsequent years marked the birth of our children: Richard Allen, now a neonatologist in New Bedford; Massachusetts, Robert James, now an economics professor in Rome who received his Ph.D. degree from Harvard mentored by Lawrence Summers (who is now the President of Harvard) and our daughter Carol Ann, a physician

and internal medicine specialist who is working with Health Care for the Homeless in Boston. We have 3 grandchildren. My wife and children, each in different ways, have been caregivers who focus on people in need including the Dinka in the southwest of Sudan, the Masai in Kenya, those in poverty at Health Care for the Homeless in Boston and individuals with HIV at my wife's Montgomery County Dennis Avenue Public Health clinic. My daughter and wife especially focus on those people that my Hungarian mother would have called "Vizes Vereb," the "wet sparrows" of the world.

I Join the National Cancer Institute, NIH

While I was completing my medical internship at the Massachusetts General Hospital, the doctor's draft was in force and many of us looked for alternatives to a two-year appointment in the Army. Although I had not seriously considered a research career, I joined the National Cancer Institute (NCI) at the NIH in 1956 following what was essentially a sojourn as a philosophy major at the University of Chicago, medical training at Harvard Medical School and a single year of medical internship. Other than the effort supported by the $50 grant, I had no research experience. Thus, it is my associates at NIH who have been my teachers. These include my mentor, Nathaniel Berlin, my collaborators, and especially my postdoctoral fellows, students and technicians who have so often inverted the conventional mentor-student relationship by providing me with ideas, by teaching me by their example how to think in innovative ways, and by converting my vague ideas into experimental realities. I believe that more than the other writers of prefatory chapters I owe any success I will discuss here, to my associates and peers within the corridors of the NIH.

A pivotal factor in my research efforts has involved an intimate association with the Clinical Center of the NIH that opened in 1953, shortly before I joined the NCI. This research hospital provides a close proximity between the laboratory and the clinical care units, thereby facilitating the development of Renaissance groups that can be productive in a full scientific range extending from fundamental laboratory-based efforts to patient-oriented clinical research. Many major challenges can only be addressed by such patient oriented clinical research that involves a real-time relationship between the patient and the physician/scientist. Patient-oriented research is necessary for the translation of fundamental laboratory insights into new approaches for the prevention, diagnosis, and treatment of human disease. In comparison with the extramural academic community, clinical research at NIH increased in relative importance in recent decades as sources of funding for patient-oriented clinical research outside of the intramural NIH research community became limited, and the research community in general moved toward the application of molecular-biological approaches to fundamental scientific questions, and away from patient-oriented clinical research. Although basic research efforts became widely distributed throughout the nation, approximately 50% of all NIH supported clinical research beds are within the NIH Clinical Center. As a result, intramural NIH has made some of its most unique contributions through translational patient-oriented research. It is a major success story, providing a model for the renewed enthusiasm for such research in the extramural research community as well.

During my first year at the NCI, I rotated through each of its clinical branches. Subsequently I was assigned to the Metabolism Service (now the Metabolism Branch)

then directed by Nathaniel Berlin. The Metabolism Branch has through its 45 years of existence been an exemplar of the type of research requiring the presence of the patient. The research efforts on the Branch has led to the discovery of new diseases, the definition of new infectious agents and through serendipitous observations the development of novel insights that have had both fundamental and clinical implications. Furthermore this type of clinical research was required to test hypotheses concerning the pathogenesis of human diseases, and finally to develop and evaluate, in clinical trials therapeutic agents that had as their scientific basis our fundamental laboratory observations. My own research has especially benefited from the analyses of certain patient-based observations that were initially paradoxical and that could not be understood without a challenge to the prevailing paradigms.

The dominant arena of my research, clinical immunology, is a field that has undergone a revolution, leading to a transition from a largely phenomenological endeavor into a deeply analytical and technical field. Movement in this field, like the growth of our children, may not appear impressive when viewed from one day to the next; however, its progress has been dramatic when viewed using the portfolio of images taken over four to five decades. Questions that could not even be asked forty-five years ago have been definitively answered. To give a sense of the dramatic progress in the field of immunology in my own scientific lifetime, I quote from my textbook, Clinical Hematology, by Maxwell Wintrobe that appeared in 1956, the year I joined the NIH:

"The function of the lymphocyte is still obscure. Because of their strategic position in lymph nodes and because they are rich in adenosinase which splits adenosine it has been

suggested that the lymphocyte is instrumental in the destruction of toxic products of protein metabolism…a role in transference of fat from intestinal epithelium to the lacteals has been denied". Of course, this view did not do justice to the contributions of Landsteiner and Chase who had demonstrated the cellular transfer of certain forms of immunity. However it did reflect the primitive understanding we had concerning the immune system especially cellular immunity at that time. The role of the thymus and of T-cells in immunity had not been defined. One could not even consider the nature of the T-cell receptor for antigen nor meaningfully discuss how antibody and T-cell diversity was generated at this time when neither the T-cell receptor nor the multichain structure of an immunoglobulin molecule had been demonstrated. By 1951 none of the primary immunodeficiency diseases had been shown to represent a defined molecular defect of an element of the immune system. Moreover, we did not have anything approaching our present understanding of a disease such as AIDS caused by a retrovirus, a form of pathogen that had not been defined, that infects CD4 expressing target T-cells that had not been discovered. In this chapter, I will start by considering certain scientific issues and experiments that were of interest to me decades ago. Then as I complete the discussion of a specific scientific issue I will fast forward the time to the present to report what has been accomplished in the particular scientific arena. In short I will consider what scientific fruits have developed from the seeds provided by early fundamental laboratory and clinical observations.

Initially after joining the NIH in 1956, I continued my interest in erythropoietin and erythropoiesis on the NCI Metabolism Service with Nathaniel Berlin. Furthermore, I was assigned to a co-mentor, Jessie Steinfeld who focused on the field of serum protein

metabolism. Jessie left the NIH shortly after I joined his laboratory and, despite my lack of research training, I became de facto a senior investigator with my own laboratory.

Erythropoietin and The Control of Erythropoiesis

For a short period I followed two scientific paths: the study of erythropoietin and erythropoiesis and the metabolism of serum proteins. In terms of erythropoietin and erythropoiesis I was lucky to have Nathaniel Berlin as my mentor and to have Wendell Rosse join me as the first medical staff fellow that I chose myself. As I have indicated to you already, and as was so often true with my associates, Wendell brought with him an interesting scientific challenge and his own novel approach to answering the scientific question: are there extra-renal sites involved in the production of erythropoietin in response to anoxia? To address this issue, we generated parabiotic rats and held them in a device that permitted the introduction of room air or hypoxic air to either one of the two joined animals whose circulations shared only a capillary connection. We nephrectomized one of the partners of the parabiotic pairs. Of interest, when only the nephrectomized partner was exposed to hypoxic air, increased erythropoiesis of the pair was observed confirming an extra-renal site of erythropoietin production. This observation became of special relevance when we examined patients who had erythrocytosis associated with non-renal tumors. We demonstrated that in patients with erythrocytosis their extra-renal tumors produced erythropoietin.[(1)] In particular, we focused on a patient, JGH, who came to the NIH complaining of a staggering gait. He had been picked up by the police who as was often the case assumed that he was drunk. This recurrent humiliation discouraged him and in his depressed state, he indicated to the police that he would go home by jumping off the Calvert Street Bridge, now renamed the

Duke Ellington Bridge, if a solution to his medical dilemma was not sought. The police delivered him to the recently established Clinical Center of the NIH. We were called and admitted him to our service where he agreed to become a participant in a study concerning the metabolism of serum proteins. However we soon recognized that he had marked erythrocytosis; that was not associated with an elevation of the other formed elements of the blood. He had von Hippel-Lindau's disease with a cerebellar hemangioblastoma causing his ataxia as well as the associated polycythemia. Wendell and I extracted this patient's cerebellar hemangioblastoma tumor, removed as required for his clinical care, and demonstrated that this tissue contained an erythropoiesis-stimulating molecule that shared the physical characteristics of erythropoietin.

In the four decades since these early studies there have been dramatic advances in the field involving erythropoietin. These include the identification, and molecular cloning of the gene encoding erythropoietin. Furthermore this normal hormone has become a major therapeutic agent for patients with anemia associated with renal disease, AIDS, or for those receiving cancer chemotherapy. In addition, in the field of von Hippel-Landau's (VHL) disease other investigators at the NCI including Berton Zbar, Marston Linehan, and Richard Klausner demonstrated that a disordered VHL gene is responsible for this disease as well as for select forms of renal cancer, and have defined its role as a suppressor oncogene.[(2)] Furthermore, the von Hippel-Landau tumor suppressor protein was shown to play a role in the regulation of the degradation of the hypoxia-inducible factor Ia, thereby providing a linkage between the genetic error in the VHL disease and the excessive erythropoietin production that lead to the erythrocytosis observed in our patient.

Entrée into Immunology through the Study of Normal and Disordered Immunoglobulin Metabolism

The dominant area of my research over the past four decades has been part of the fabric of immunology that is dedicated to the understanding of the complex of interacting cells and antibodies that protect us from infection. My own entry into this field was through the back door, by the study of serum protein metabolism and turnover. Initially the focus of our studies was on the metabolism of albumin in normal individuals and those with hypoalbuminemia due to either reduced synthesis or alternatively to a short protein survival that we showed in turn was associated, in many cases, with protein-losing enteropathy. We soon turned from albumin metabolism to an examination of the metabolism of the five major classes of immunoglobulins. We demonstrated that they have different rates of synthesis, patterns of distribution, and rates of catabolism in normal human individuals.(3) Furthermore, we found that naturally occurring and synthetically produced Fc immunoglobulin fragments have metabolic characteristics that are comparable to those of the parent molecule, whereas light chains and Fab fragments are far more rapidly catabolized and appear to be handled by renal metabolic pathways that are different from those of the whole molecule. We indicated that the metabolism of such small immunoglobulin fragments involves passage through the glomerulus and then catabolism in the proximal convoluted tubule of the kidney. The studies of the pharmacokinetics of the five immunoglobulin classes and their subunits provided the scientific basis for the dosing schedules that are now used in the development of rational regimens that employ monoclonal antibodies and their fragments in the treatment of human disease.

One of the physiological factors that controls the IgG catabolic rate as well as its specific saturable transport across the neonatal gut of the mouse and the placenta of humans was shown by Brambell to involve the IgG concentration.[4] We also demonstrated a direct relationship between the serum IgG concentration and its fractional catabolic rate in humans. A high serum IgG concentration was associated with a short IgG survival.[3] In particular, in humans the fraction of the intravascular pool of IgG catabolized daily, rose from 2% in patients with hypogammaglobulinemia associated with decreased IgG synthesis to an upper asymptomatic limit of 16 to 18 percent in patients with multiple myeloma and associated serum IgG concentrations over 30 mg/ml.[3] Brambell suggested that this concentration-catabolism effect could be explained by postulating a saturable carrier-mediated protection system specific for IgG molecules.[4] Similarly, the transport of immunoglobulins from the mother to the fetus or newborn appeared to utilize a saturable IgG specific process that could be competitively inhibited by the addition of homologous or heterologous IgG or its Fc fragment. In the early 1970's we reexamined this issue and demonstrated that receptors could be extracted from the rat neonatal intestine and from the adult carcass. These receptors bound IgG molecules and had the characteristics of an IgG specific receptor involved in both the normal perinatal transport, as well as the concentration-catabolism effect involving this immunoglobulin class.[5] In 1989 using biochemical and molecular techniques Simister and Mostov [6] demonstrated that this IgG specific transport and catabolism receptor was a heterodimer involving the ∃2 - microglobulin light chain that was associated with an IgG Fc specific receptor structurally related to MHC class I molecules.

During the subsequent years we applied our analyses of immunoglobulin metabolism to the study of the pathogenesis of the reduced serum immunoglobulin concentrations in patients with different forms of hypogammaglobulinemia. We demonstrated that such disorders of immunoglobulin concentration may occur secondary to a variety of pathophysiological mechanisms that include decreased synthesis of all classes of immunoglobulin or reduced synthesis of only one or two immunoglobulin classes. In one of our early contributions we defined additional new categories of immunoglobulin deficiency diseases caused by defects of endogenous protein catabolic pathways or loss rather than by abnormalities of immunoglobulin synthesis. [3, 7-9] For example, we demonstrated that hypogammaglobulinemia may be the result of hypercatabolism affecting a single immunoglobulin class such as we observed in patients with myotonic dystrophy where there is a selective short survival of IgG .[7] In other patients the short survival of a particular immunoglobulin class was due to the development of circulating antibodies to that class as was observed in select patients with IgA deficiency who had developed an anti-IgA antibody. Alternatively, we showed that endogenous hypercatabolism may affect all immunoglobulin classes and albumin, as we reported in patients with what we called "familial hypercatabolic hypoproteinemia," a previously unreported syndrome that involved reduced protein serum concentrations associated with reduced survivals of diverse immunoglobulin classes and albumin.[8]. Our group in collaboration with Robert Gordon, discovered yet another form of disordered protein metabolism leading to hypogammaglobulinemia, in this case, associated with the excessive bulk loss of serum proteins into the gastrointestinal tract by what we called "protein-losing gastroenteropathy".[9] We demonstrated that diverse patients previously

diagnosed as having idiopathic hypoproteinemia, had protein-losing gastroenteropathy as the pathophysiological mechanism leading to their hypoproteinemia.[9] We developed two of the techniques that permitted the identification and quantitation of such gastrointestinal protein loss, the ^{67}Cu-ceruloplasmin and ^{51}Cr labeled albumin clearance tests.[10] Protein-losing enteropathy was shown by our group and by others to occur as an associated feature of over one-hundred disorders that affect the gastrointestinal tract.[9] Through the analysis of these disorders we were able to define a number of new clinical syndromes that included "allergic gastroenteropathy" where there is an abnormal response to milk and the syndrome "intestinal lymphangiectasia" when our attention, in hypoproteinemic patients, was directed towards the intestinal tract by the use of radiolabeled protein clearance techniques.[11] Intestinal lymphangiectasia that we defined four decades ago was shown by our group to represent a generalized disorder of the development of lymphatic channels including those of the gastrointestinal tract (termed Waldmann's Disease by the National Organization for Rare Disorders). In conjunction with my associate Warren Strober, we demonstrated that in intestinal lymphangiectasia there is the gastrointestinal loss of both serum proteins and lymphocytes a phenomenon that represents the pathological equivalent of a thoracic duct fistula and that causes a novel form of immunodeficiency characterized by profound hypogammaglobulinemia, lymphocytopenia, skin anergy, and impaired homograft rejection.[12] Both sporadic and familial cases of intestinal lymphangiectasia were defined. By way of an update on this syndrome, the VEGF R-3, a receptor tyrosine kinase known to be pivotal for normal lymphatic development, was shown to be defective in some patients with a familial, generalized lymphatic abnormality[13]

Immunoregulatory Disorders Defined though the Study of Patients who have Genetic Immunodeficiency Diseases; The Demonstration that Intrinsic B-cell Defects, Helper T-cell Disorders or Excessive Suppressor T-cell Activity Underlie the Immunoglobulin Deficiency in Patients with Primary Immunodeficiency Diseases

As noted above, we used an analysis of disorders of immunoglobulin metabolism as our entrée into the field of immunology, but soon enlarged our focus to involve the study of the pathogenesis of the immunological disorders of patients with hereditary abnormalities in their levels of immunoglobulins as well as cellular elements of the immune system. We initially focused on these rare but instructive patients, to help define the stages in the sequential development of bone marrow stem cells into mature effectors of the immune system, and to obtain insights into the pathogenic role played by disorders in the network of interacting regulatory T-cells that normally control the immune response.

During the late 1950's and early 1960's after I joined the NIH, the understanding of immunology expanded rapidly as the result of experimental work in whole animals and the study of human patients who either had a lymphocytic leukemia or one of the hereditary primary immunodeficiency diseases. The analysis of these latter patients, with experiments of nature, in which one or another essential component of body defenses was missing or aberrant led to the recognition that disordered immune responses could reflect either intrinsic defects in the development and function of B lymphocytes or alternatively abnormalities of the regulatory network involving either helper or suppressor T-cells. In the course of our studies of immunoglobulin metabolism, we

identified patients with an array of disorders associated with defective immunoglobulin synthesis. In an effort to define the pathogenic mechanisms underlying these synthetic defects we and others developed techniques to analyze the terminal differentiation of human B lymphocytes examined ex vivo by the stimulation of these cells with lectins such as pokeweed mitogen to induce the B cells, in the presence of T-cells, to become immunoglobulin synthesizing and secreting cells. In addition, we developed co-culture techniques to assist in identifying disorders of the helper and suppressor T-cells that normally regulate B-cell maturation by facilitating or inhibiting this process. [14] In the majority of patients, the hereditary immunoglobulin deficiency was associated with an intrinsic B-cell defect. Within this broad group we were able to identify new immunodeficiency syndromes including X-linked hypogammaglobulinemia associated with isolated growth hormone deficiency, a syndrome that could be distinguished from Bruton's X-linked agammaglobulinemia that, in turn, is associated with a Btk tyrosine kinase abnormality.[15] In our co-culture studies the mononuclear cells of some groups of patients with immunodeficiency but with normal B-cells were shown to manifest a helper T-cell defect. In particular a helper T-cell abnormality was the major abnormality in patients with X-linked hyper-IgM. The B-cells of such patients could produce IgM molecules but could not accomplish a normal immunoglobulin class switch. The addition of irradiated normal T-cells ex vivo to the peripheral blood B-cells of such patients allowed them to produce additional classes of immunoglobulin molecules in the pokeweed mitogen stimulated in vitro biosynthesis co-culture system. In a collaborative effort, Lloyd Mayer demonstrated that the lymphocytes of such patients with the

X-linked form of hyper-IgM would synthesize the other immunoglobulin classes when the patients' cells were co-cultured with the "switch" T-cells from a patient with the helper T-cell leukemia—the Sézary syndrome. [16]

Subsequently a number of other groups demonstrated that patients with the X-linked hyper-IgM syndrome have a defect in their expression of the CD40 ligand (CD40L) on activated helper T-cells that normally are involved in the immunoglobulin class switch manifested by B lymphocytes. Helper T-cell disorders along with B-cell defects also contributed to the dysgammaglobulinemia that characterizes the ataxia telangiectasia syndrome. Such patients have an immature thymus gland that lacks Hassall's corpuscles. It had been proposed that this failure of thymus development was secondary to an abnormality of the interactions between the mesodermal and entodermal anlage that are necessary for the maturation of this organ. I reasoned that a similar failure of organ development might lead to immaturity of the liver, an organ that also requires these interactions. To address this hypothesis we established a radioimmunoassay for alpha fetoprotein and reported an elevation of this fetal protein in all of the patients with ataxia telangiectasia we studied.[17] The establishment of this radioimmunoassay along with one for human chorionic gonadotropin (HCG) permitted us to make a segue into tumor immunology that involved our introduction of these assays into the evaluation of patients undergoing therapy for germ cell tumors of the testis. In a series of studies in collaboration with different clinical trial groups we showed that the two radioimmunoassays taken together provided valuable surrogate markers of disease activity[18] Persistence of an elevated alpha fetoprotein or HCG level indicated the presence of residual tumor and predicted a recurrence in those patients who do not

receive further chemotherapy.[18] The application of these tests was of critical value in the subsequent development of curative chemotherapeutic regimens for germ cell tumors.

Antigen Non-specific Suppressor T-cells

Stimulated by Richard Gershon's discovery of antigen specific suppressor T-cells in mice, I considered the possibility that a subset of patients with common variable hypogammaglobulinemia might have aberrant antigen non-specific suppressor T-cell activity as a pathogenic factor underlying their broad immunodeficiency. When the immunoglobulin biosynthesis in vitro coculture assays were applied to the peripheral blood mononuclear cells of patients with common variable immunodeficiency, the majority of patients with this disorder were shown to have intrinsic B-cell defects. However in another subset involving 10 to 15 percent of the patients, there were excessive numbers of circulating activated suppressor T-cells that inhibited the B-cell maturation and immunoglobulin synthesis of co-cultured normal peripheral blood mononuclear cells.[14] The B cells of the patients in this latter subset were able to synthesize IgM normally when freed ex vivo of their suppressor T-cells, but could not do so when their own T-cells were returned to the cultures of the patients' own mononuclear cells. These studies represented the first demonstration of aberrant activated T-cell mediated suppression of an immune response in humans, thus changing the way investigators thought about the pathogenesis of certain immunological disorders. In the intervening years since these early efforts to understand immunodeficiency diseases where they were viewed as disorders of the pathways of B-cell differentation and maturation and their interactions with regulatory T-cells, over 75 molecular genetic defects have been defined that not only provide major insights concerning the

pathogenesis of immunodeficiency diseases but also define the role of different immunological cells and their interactions in the normal immune response. These more recent discoveries by others, have also provided new approaches to the diagnosis including intrauterine diagnosis of immunodeficiency diseases. Furthermore, the definition of the underlying genetic defects provided the scientific basis for rational therapeutic strategies that included the first successful approach to gene therapy for one of these genetic disorders, that of the X-linked severe combined immunodeficiency disease (SCID) that is associated with a disorder of the common gamma chain ((c).[19]

The Study of Human Leukemias with Retained Functions Provided Insights into the Regulatory Network of T-cells

Although the studies of genetic primary human immunodeficiency diseases were of great heuristic value, our understanding of the immune system was hindered by the fact that unseparated lymphocytes represent mixtures of complex populations of cells with different and at times opposing functions. They presented a confusing Tower of Babel sending diverse and often conflicting signals that were difficult to analyze. Monoclonal antibodies and the techniques to clone lymphocytes had not been developed. To circumvent this problem Samuel Broder my associate, and I took advantage of the fact that in a given patient human lymphoid leukemic cells represent homogenous populations of T-cells that could theoretically retain a single function. Using the ex vivo immunoglobulin biosynthesis co-culture assay we were able to show that some leukemic T-cells, in particular those of patients with the Sézary syndrome, can act as helper T-cells when co-cultured with normal B-cells. In contrast, the T-cells of patients with human T

cell lymphotropic virus I (HTLV-I) associated adult T cells leukemia (ATL) profoundly inhibited immune responses by functioning as immune suppressors in such co-cultures. [20, 21] Our patients with this latter leukemia were profoundly anergic and were unable to make either antibody responses or skin test responses to recall antigens. Effective therapy of these patients with adult T-cell leukemia was associated with a return to normal immune function in vivo. In the period following the publications of our studies on suppressor T-cell abnormalities in common variable hypogammaglobulinemia and adult T-cell leukemia, concern was expressed about our focus on such suppressor T-cells as pathogenetic factors underlying the immunodeficiency state. This in part reflected the difference in the scientific interest of those investigators focusing on the immune responses of mice, and our interest focusing on those of humans. Those scientists focusing on mice in their analysis of immune responses were especially interested in antigen specific, genetically restricted immune responses whereas our studies focused on genetically nonrestricted, antigen nonspecific events mediated by suppressor elements. With the decline in acceptance of antigen specific suppressor cells in mice there was a parallel loss of interest in human suppressor T-cells. However, recently, there has been a major reawakening in the acceptance of antigen nonspecific suppressor T-cells with the definition of the importance, in mice, of a population of CD4+, CD25+ (IL-2R∀) suppressor or negative regulatory T-cells.[22] It should be noted that in 1984 we reported that the HTLV-I associated adult T-cell leukemia cells of our patients both functioned as profound suppressor cells, and had the phenotype CD4+, CD25+ the phenotype that has now been identified as that of the negative immunoregulatory cell.[21] Furthermore, more recently in conjunction with Thomas

Fleisher of the Clinical Center NIH we demonstrated that these ATL cells have the full phenotype characteristic of the suppressor cells that have recently been the focus of is reawakened interest. In particular, ATL T-cells manifest the CD4+, CD25+, CD62L, high CD45RO+ and CTLA4+ phenotype. It is our hypothesis that the HTLV-I associated ATL cells that we reported functioned as suppressor cells in our ex vivo co-culture studies in the early 1980's represent a human leukemic T-cell expansion of the recently reaccepted CD4+, CD25+ negative immunoregulatory T-cell. Taken as a whole these studies of human immunodeficiency diseases and T-cell leukemias have provided valuable insights into the complex regulatory network of cells that controls the human immune response.

Molecular Genetic Analysis of Immunoglobulin and T-Cell Receptor Genes in Human Lymphoid Neoplasms

Until the mid-1970s, the cardinal question regarding the immune system remained unanswered: How does our body with its limited amount of genetic material generate a diversity of antibodies and T-cells that can recognize a myriad of foreign configurations in our environment? The solution to this paradox emerged from the brilliant studies of Tonegawa, Leder, and Hood who used recombinant DNA technology to show that the genes encoding antibodies and those of T-cell receptors utilize discontinuous bits of genetic material that like letters of the alphabet can be shuffled and rearranged into many combinations to yield diverse sequences, a mechanism underlying one component of the observed immunoglobulin and T-cell receptor diversity. Phillip Leder, aware of our interest in the hereditary immunodeficiency diseases, suggested a collaboration to examine the immunoglobulin genes of such patients. However, the disordered genes

associated with the majority of primary immunodeficiency diseases and those encoding the immunoglobulin molecules were present on different chromosomes. As an alternative subject for study we correctly reasoned that Southern blot analysis of immunoglobulin gene rearrangements could serve as tumor-specific clonal markers capable of detecting even minority populations of monoclonal cells in patients with different forms of lymphocytic leukemia. These studies, the initial ones, applying immunoglobulin gene rearrangement analysis to human cells have had wide ranging implications for the diagnosis and the monitoring of therapy of B and T-cell neoplasms. Stanley Korsmeyer, Phillip Leder and I used this analysis of receptor gene rearrangements to define the lineage (T or B cell) of leukemic cells lacking conventional markers, to establish whether the abnormal lymphocytic populations were polyclonal, oligoclonal, or monoclonal, to determine the state of maturation of leukemic B and T-cell precursors and to broaden the scientific basis for the diagnosis and monitoring of the therapy of lymphoid neoplasms.[23] For example, we established that the majority of what had been referred to as the non-T/non-B form of acute lymphoblastic leukemia represented a developmental series of B-cell precursors. Furthermore, this analysis was the first to establish a hierarchical order of immunoglobulin gene rearrangements in humans wherein heavy chain gene rearrangements precede those of light chains and kappa – gene rearrangements precede lambda gene rearrangements.

Interleukin-2 and Its Receptor

The final stops in my odyssey of immunological reminiscence will be on the critical roles played by the cytokines, Interleukin-2 (IL-2) and Interleukin-15 (IL-15)

and their receptors, on the growth and differentiation of normal and neoplastic T-cells. Furthermore, it will be on the translation of the basic insights concerning the IL-2 and IL-15 systems into receptor-directed, monoclonal antibody – mediated strategies for the treatment of patients with leukemias and lymphomas, those with autoimmune diseases as well as for the prevention of allograft rejection. Our work on the IL-2 receptor emerged during a halcyon period for our group, a moment two decades ago when within our laboratory, we were joined in a team effort by my associates, Takashi Uchiyama, Stanley Korsmeyer, Warner Greene, Warren Leonard, Andrew Arnold and by Carolyn Goldman who has been my indispensable coworker for over 25 years. In the early 1980s, when these studies were initiated, the HIV virus had not been identified, the T-cell antigen receptor had not been cloned, and our understanding of the regulation of the immune system and its function was quite different from that of today. Our efforts became focused on the question, How do T-cells grow and develop effector functions following activation? Takashi Uchiyama joined the laboratory shortly after Köhler and Milstein's development of hybridoma technology, and their initial production of monoclonal antibodies captured the imagination of biomedical scientists. Uchiyama's production of the anti-Tac monoclonal directed toward the IL-2 receptor alpha subunit was a classic example of serendipity.[24] He used a T-cell line, we developed from a patient CR who carried the diagnosis of Sézary T-cell leukemia, but who in retrospect had HTLV-I associated adult cell leukemia, as a target in our efforts to produce an anti-CD4 antibody during a period when such antibodies were being embargoed and were not made available to the scientific community. The antibody anti-Tac (T-cell activation antigen) we produced however did not target the CD4 antigen, rather it reacted with what we now

recognize as the alpha subunit of the receptor for Interleukin-2 (then termed T-cell growth factor) that had just been discovered by Morgan, Ruchetti, and Gallo.[25] At that time the mode of action of IL-2 was undefined; that is which cells expressed its receptor, and under what conditions. Warren Leonard and Warner Greene joined our laboratory and determined that anti-Tac blocked the ability of IL-2 to stimulate T-cells to divide.[26] This was an important finding since anti-Tac was one of the first, if not the first monoclonal antibody to define a receptor for one of the cytokines that immune cells use to communicate with one another. We biochemically characterized the receptor peptide identified by anti-Tac as a densely glycosylated, sulfated, integral membrane protein with an apparent Mr of 55,000. Along with two other groups, using the anti-Tac monoclonal antibody to purify the receptor peptide, Warren Leonard and other members of our group succeeded in cloning, sequencing and expressing cDNAs encoding the 33 kD polypeptide backbone of the 55kD IL-2 receptor protein.[27] Based on this DNA sequence, the primary structure of this IL-2R∀ receptor peptide was shown to be composed of 272 amino acids including a 21 residue signal peptide and a short 13 amino acid intracytoplasmic domain that was too short to function in signaling. This raised the issue of how this receptor's signals were transduced to the nucleus. Furthermore, questions were posed concerning the IL-2 receptor that were difficult to answer when only the 55 kDa IL-2R∀ peptide was considered. These questions included: What was the structural explanation for the great difference in affinity between high (10^{-11}M) and low affinity (10^{-8}M) receptors? How could certain Tac non-expressing cells including resting natural killer cells respond to IL-2? In association with Mitsuru Tsudo, who had joined our laboratory, we resolved these issues, in parallel with investigators in the Leonard laboratory by codiscoveing a

novel non-Tac IL-2 binding protein, IL-2R∃, with an Mr of 75, 000.[28] We proposed a multi-chain model for the high affinity IL-2 receptor. Subsequently, Sugamura and coworkers discovered the IL-2R(chain or (c that is required for high affinity IL-2 binding and signaling.[29] In subsequent studies, we demonstrated that the alpha chain of the IL-2 receptor identified by the anti-Tac monoclonal antibody is not expressed by the majority of normal resting cells but is expressed by the abnormal T-cells of patients with a wide range of diseases including different forms of human leukemia, the T-cells involved in the pathogenesis of autoimmune disorders, as well as by the T-cells participating in organ allograft rejection. This discovery represented a major turning point for our clinical efforts in that it suggested that we could exploit the difference in IL-2 receptor expression between normal cells and T-cells by designing IL-2R directed agents to eliminate unwanted CD25 expressing T-cells and thereby treat human disease. Our developments of IL-2R alpha and IL-2R beta directed monoclonal antibody strategies that dominate our clinical trails efforts are considered below.

Interleukin-15

The most recent scientific pathway in the immunological odyssey of our lab was initiated with our co-discovery of the cytokine interleukin-15.[30] This discovery emerged from a paradoxical observation that was difficult to explain with the paradigms of the time, that was made during our IL-2 receptor-directed clinical trials that involved patients with HTLV-I associated adult T-cell leukemia (ATL). In the early phases of ATL, the leukemic cell proliferation is linked to an autocrine process involving the coordinate expression of IL-2 and IL-2R∀. PHowever in late phase ATL, the leukemic cells no longer produce IL-2 yet continue to express increasing numbers of cell surface IL-2

receptors. As part of our studies of this IL-2 – independent phase of the ATL leukemic cell proliferation, we focused on HuT-102 an ATL cell line that was derived from patient CR and that manifested over 100,000 IL-2Rs per cell yet did not express mRNA encoding IL-2. We showed that the supernatants from cultures of this cell line stimulated the proliferation of an indicator cytokine dependent cell line, CTLL-2. Furthermore this stimulatory action was not inhibited by the addition of an antibody to IL-2 but was associated with a previously undefined 14-15kDa lymphokine that required expression the IL-2R∃ subunit for its stimulation of T-cell proliferation and for its induction of NK cell activation.[30] Grabstein and coworkers[31] simultaneously reported the recognition of this cytokine, now known as IL-15, which they isolated from supernatants of a simian kidney epithelial cell line CV-1/EBNA. With the use of an antibody to IL-15 we demonstrated that our factor and that of Grabstein's were identical. The cDNA defining IL-15 encodes a 162aa peptide with a 48aa leader sequence yielding a 114aa mature protein. Our co-discovery of IL-15 emphasizes the important role played by translational research wherein observations made using material derived directly from patients can open new basic science arenas. It is of note that the HuT-102 cell line used in these studies to identify IL-15 was from the patient CR discussed above who had adult T-cell leukemia. Cell lines from this same patient were utilized by Poiesz and coworkers[32] to discover the first pathogenic human retrovirus, HTLV-I , and were the target cells that we used in the generation of the anti-Tac monoclonal antibody that first defined an IL-2 receptor subunit.[24] and that, as discussed below, has been used in our IL-2 receptor directed clinical trails.

We helped to define two distinct receptor and signaling pathways that are used by IL-15 in diverse cells. As predicted from the ability of IL-15 to stimulate the proliferation of the putatively IL-2 specific-cytokine-dependent CTLL-2 cell line we demonstrated that IL-15 uses a multi-subunit receptor in T- and NK-cells that involves the IL-2R∃ chain shared with IL-2, as well as the common (c subunit shared with IL-2, IL-4, IL-7, IL-9, and IL-21.[30] Giri and coworkers [34] demonstrated that the high affinity IL-15 receptor also includes a private IL-15R∀ receptor element. In an effort to define functions mediated by IL-15 and not shared with IL-2, Yutaka Tagaya of our group focused on mast cells that do not respond to IL-2 since they lack the required IL-2R∃ chain.[33] He postulated that if mast cells responded to IL-15 that this would be an indication that IL-15 utilizes non-IL-2R components in these cells. In accord with his prediction, we demonstrated that IL-15 is a mast cell growth factor and that its signaling in such cells involves a receptor system that does not share any subunits with the IL-2/IL-15 receptor system of T-cells. Rather this mast cell receptor uses a 60-65kDa novel receptor, IL-15RX. Furthermore, this receptor employs a signal transduction pathway involving Jak-2 and STAT-5 in contrast to the Jak-1/Jak-3 and STAT-3/STAT-5 pathway used by the IL-2/IL-15R system in T-cells.

The Contrasting Roles of IL-2 and IL-15 in the Life and Death of Lymphocytes

We compared and contrasted IL-2 and IL-15 mediated functions in terms of the fundamental goals of the immune system, that in a simplified form include: (a) the generation of a rapid innate (e.g., NK cell) and adaptive (e.g. antibody and T-cell) immune responses to invading pathogens, (b) the maintenance of a specific memory response to these pathogens, and (c) the elimination of autoreactive T-cells to yield

tolerance to self. As might be anticipated from their shared use of the IL-2R∃ and (c subunits in T and NK cells, we and others, demonstrated that IL-15 and IL-2 share a number of functions including the stimulation of T-cell proliferation, activation of NK cells, and the induction of immunoglobulin synthesis by human B-cells co-stimulated with anti-IgM or anti-CD40. Furthermore a special role for IL-15 has been demonstrated in the development of NK cells, as well as in the development and persistence of memory phenotype $CD8^+$ cells. Yutaka Tagaya a coworker prepared transgenic mice expressing IL-15. These mice manifested a major increase in the number of their NK cells, NK-T-cells and CD44hi, $CD8^+$ memory phenotype T- cells.[35]

A major advance emerging from our laboratory and those of others is that although IL-2 and IL-15 share two receptor subunits and some functions, they provide distinct and at times competing contributions to other aspects of the life and death of lymphocytes.[35-38] Although IL-2 is an important growth and survival factor, it also plays a pivotal role in FasL-mediated activation induced cell death (AICD) of CD4 T-cells. Receptor-mediated stimulation of CD4 T-cells by antigen at high concentration induces the expression of both IL-2 and the IL-2 receptor, which, in turn, interact to yield T-cell activation and cell cycling. Antigen restimulation of the cycling T-cells at this stage through the T-cell antigen receptor increases the transcription and expression of the death effector molecule FasL. We demonstrated that IL-15, in contrast to IL-2, acts to extend the survival of lymphocytes both by acting as a growth factor and by inhibiting IL-2 mediated $CD4^+$ T-cell AICD.[35, 37] In particular, in ex vivo studies, CD4 T-cells from the IL-15 transgenic mice that Yutaka Tagaya developed did not

manifest IL-2 mediated AICD.[35] In addition to their distinct actions in AICD, IL-2 and IL-15 play opposing roles in the control of the homeostasis of $CD8^+$ memory phenotype T-cells.[35-38] Again IL-15 is involved in the maintenance of T-lymphocyte survival whereas IL-2 has the opposite effect leading to their loss. Ku and coworkers[36] reported that the division of $CD8^+$ T-cells of memory phenotype is stimulated by IL-15, but is inhibited by IL-2. We, in turn, demonstrated that the IL-15 transgenic mice have abnormally elevated numbers of $CD8^+$ memory phenotype T-cells.[35] Furthermore, we defined a role for IL-15 and its beta receptor in HTLV-I associated ATL and the neurological disease tropical spastic paraparesis (TSP).[38] The number of circulating MHC Class I (A 201) restricted antigen specific (amino acids 11-19 of the HTLV-I encoded tax protein) reactive $CD8^+$ cells that have been suggested to be involved in the pathogenesis of TSP were shown by Nazli Azimi of our group and Steven Jacobson using tetramer technology, to be markedly increased in the circulation of patients with TSP.[38] Azimi monitored the survival of such CD8 antigen specific T-cells ex vivo in the presence or absence of antibodies to the cytokines IL-2 and IL-15 or to their receptors and thereby demonstrated that the addition of antibodies to IL-15 or to its receptor beta subunit to such ex vivo cultures led to the rapid (within the six days) reduction in the number and function of the antigen specific memory phenotype $CD8^+$ cells whereas the addition of antibodies to IL-2 or to its private IL-2R alpha receptor did not have this effect. Taken together, the studies support the view that in their special adaptive immune functions, IL-2 and IL-15 favor opposing actions that emphasize one or the other of the two competing major goals of the immune response. IL-2 through its contribution to AICD for $CD4^+$ cells and its interference with the persistence of memory $CD8^+$ phenotype

T-cells, favors the elimination of those lymphocytes that are directed toward self antigens and thus IL-2 plays a critical role in the maintenance of peripheral self-tolerance. In contrast, IL-15 through its inhibition of IL-2 mediated AICD and its positive role in the maintenance of $CD8^+$ memory phenotype T-cells, favors the maintenance and survival of T-cells that are of value in providing a long term specific memory immune response to foreign pathogens. In accord with this role for IL-15, the elimination of vaccinia virus was more effective in our IL-15 transgenic mice than in wild-type mice.[35] An analysis of IL-2 and IL-2R∀ as well as of IL-15 and IL-15R∀ knockout mice support these conclusions concerning the competing roles for IL-2 and IL-15 in AICD and in the expression of memory phenotype $CD8^+$ cells. [37]

The Regulatory Controls Affecting IL-15 Expression

The uncontrolled expression of IL-15 carries with it the risk to the organism of the survival of auto-reactive T-cells that could lead to the development of autoimmune diseases. As just noted, IL-15 is a potentially dangerous inflammatory cytokine in that it inhibits self-tolerance mediated by AICD, facilitates the persistence of $CD8^+$ memory T-cells and induces the expressions of TNF∀, IL-1∃ and inflammatory chemokines. In terms of the regulation of cytokine expression, IL-2 is produced by activated T-cells and its synthesis is controlled at the levels of mRNA transcription and stabilization. In contrast we have defined a complex multifaceted regulation of IL-15 expression.[39-42] IL-15 is not produced by T-cells, but there is widespread expression of IL-15 mRNA in a wide variety of tissues and cells. Using interferon regulatory factor-1 (IRF-1) knockout mice, in conjunction with the Laboratory of Tadamitu Tangiguchi, IRF-1 was shown to be a critical element in the induction of IL-15 transcription.[42] Furthermore, we

demonstrated that the HTLV-I tax protein transactivates IL-15 transcription through the action of NF-kB. Nevertheless, the regulation of IL-15 expression is predominantly post-transcriptional and occurs at the levels of IL-15 mRNA translation and protein trafficking and translocation within the cell. In particular, although IL-15 message is widely expressed constitutively it has been difficult to demonstrate the IL-15 cytokine in the supernatants of many cells that express such message. [39-41] In particular, we observed that although monocytes activated with LPN/IFN(express high levels of mRNA encloding IL-15, culture supernatants from these cells did not contain sufficient IL-15 to be identified by either an IL-15 specific ELISA or by the CTLL-2 proliferation assay.[39] This demonstration of a discordance between IL-15 message expression and IL-15 protein secretion led us to examine normal IL-15 mRNA for post-transcriptional controls particularly for features that would impede IL-15 production at the level of mRNA translation. We demonstrated that the IL-15 message includes a number of elements that are impediments to its translation. In particular, the 5' UTR of the normal human IL-15 message is burdened with 13 upstream AUGs that interfere with efficient IL-15 translation. Furthermore, the unusually long 48 amino acid signal peptide sequence and a cis-acting element in the 3' mature protein-coding region interfere with this process. Taken together these observations suggest that IL-15 mRNA unlike IL-2 mRNA may exist in translationally inactive pools. As a hypothesis, we propose that by maintaining a pool of translationally inactive IL-15 mRNA mononuclear cells may respond to an intracellular infectious agent by unburdening the IL-15 message thereby transforming it into one that can be efficiently translated, thus yielding IL-15 that would facilitate the activation of NK and T-cells that could clear the pathogen. Our current

efforts are directed toward defining the putative molecular events involved in unburdening the IL-15 message thereby facilitating its translation.

Disorders of IL-15 Expression in Patients with Autoimmune Diseases and Disorders Associated with the Retrovirus HTLV-I

Despite the complex regulation of IL-15, abnormalities of IL-15 expression have been described in patients with rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, autoimmune chronic liver disease, T-cell mediated alveolitis in with diseases associated with the retroviruses HIV and HTLV-I. Our group has had a special interest in the study of HTLV-I associated tropical spastic parasparesis (TSP). In particular, T-cells from patients with TSP manifest spontaneous proliferation in ex vivo culture in the absence of exogenously added cytokines. We demonstrated that this proliferation is, in part, due to the transactivation by the HTLV-I encoded tax protein of the expression of IL-2 and IL-2R∀ since addition of antibodies to IL-2 or its private receptor subunit partially inhibited the proliferation.[43] This coordinate expression of IL-2 and its receptor established an autocrine loop, which participates in the abnormal proliferation of HTLV-I infected T-cells. However, this observation left open the question of why the blockade of the interaction of IL-2 with its receptor in TSP did not completely abrogate the ex vivo mononuclear cell proliferation. In studies to address this question, we demonstrated that the HTLV-I encloded tax protein also transactivates the transcription of both the IL-15 and IL-15R∀ genes through a mechanism involving an NF-kB site. The addition of antibodies to IL-15 or directed toward the IL-2R∃ receptor that is shared by IL-15 and IL-2, partially inhibited the spontaneous proliferation of peripheral blood

mononuclear cells from patients with TSP, in a fashion similar to the parallel observation with antibodies to IL-2 or to its private receptor. When a combination of both anti-IL-2 and anti-IL-15 antibodies was used, there was a dramatic inhibition of the ex vivo proliferation observed suggesting that both cytokines contribute to the spontaneous proliferation observed in TSP. [44] We proposed a model in which at least two autocrine loops involving IL-2 and IL-15 and their receptors are active in HTLV-I infected T-cells, and contribute to their abnormal proliferation. Furthermore, as noted above, disordered IL-15 expression in TSP plays a role in the persistence of MHC-I restricted, antigen-specific $CD8^+$ memory and effector T-cells that are thought to play a role in the pathogenesis of the disease. As noted below we are planning to use the monoclonal antibody HuMik∃1 that blocks IL-15 interaction with IL-2/15R∃, thereby inhibiting IL-15 function to exploit these observations concerning its role in the pathogenesis of this autoimmune, neurological disease.

The IL-2/IL-15 Receptor Systems: Targets for Immunotherapy in Patients with IL-2R or IL-15R Expressing Leukemia/Lymphomas, Autoimmune Diseases and to Prevent Allograft Rejection

The Food and Drug Administration on December 10, 1997 approved daclizumab or Zenapax^R (the humanized version of our anti-Tac monoclonal antibody) for use in humans to prevent acute kidney transplant rejection. This was the first humanized monoclonal antibody approved for use in transplantation. Furthermore, it was the first antibody that was directed to a cytokine or interleukin receptor to be approved. The FDA decision marked the culmination of a 16-year odyssey in our laboratory that was

responsible for a series of discoveries that laid the scientific foundation for the randomized clinical trials that ultimately led to the approval of daclizumab.

As noted above, one of our group's most critical contributions was the recognition that the IL-2 receptor represents an extraordinarly useful therapeutic target for monoclonal antibody action. [45-47] Although Köhler and Millstein's development of hybridoma technology had rekindled interest in the use of antibodies to treat patients, the initial use of monoclonal antibodies as therapeutic agents was relatively disappointing. "The magic bullet" of antibody therapy that had been the dream of immunotherapists since the time of Paul Ehrlich proved to be elusive. Nevertheless recently, monoclonal antibody-mediated therapy has been revolutionized by advances such as genetic engineering to create less immunogenic, more effective agents with better pharmacokinetics, by the arming of such antibodies with toxins or radionuclides to enhance their effector functions, and most critically by the definition of new surface structures on cancer and other cells such as growth factor and death pathway receptors as targets for effective monoclonal antibody action. Since 1981, we have focused our therapeutic efforts on the use of monoclonal antibodies directed toward the receptors for the lymphokines IL-2 and IL-15. [45-51] The scientific basis for this approach emerged from our application of the anti-Tac monoclonal antibody to the analysis of IL-2R∀ expression by normal and abnormal cells. We demonstrated that the majority of resting T-cells and monocytes do not display the alpha subunit of IL-2 receptor identified by anti-Tac. In contrast to this lack of IL-2R∀ expression by most normal resting cells, this receptor subunit is constitutively expressed by the abnormal cells in certain forms of lymphoid neoplasia including HTLV-I associated ATL, cutaneous T-cell lymphoma, anaplastic large cell lymphoma, hairy cell

B-cell leukemia and Hodgkin's disease, as well as by the activated T-cells in an array of autoimmune diseases such as T-cell mediated uveitis and HTLV-I associated tropical spastic paraparesis (TSP) and by the T-cells of individuals undergoing allograft rejection.[48, 49] We took advantage of this difference in IL-2R∀ expression between most normal resting cells and T-cells in our strategies to eliminate abnormal, activated T-cells. In the next phase of these efforts, we translated the fundamental insights from the laboratory and validated them in preclinical animal studies. In particular, in collaboration with Robert Kirkman of Harvard Medical School, we demonstrated efficacy of anti-IL-2R∀ directed monoclonal mediated antibody therapy in the prevention of renal allograft rejection in cynomolgus monkeys. In our own laboratory, we showed that the murine version of the anti-Tac monoclonal antibody delayed cardiac allograft rejection in cynomolgus monkeys.[50] The first clinical trials of murine anti-Tac were directed toward the treatment of patients with adult T-cell leukemia the malignancy of mature $CD4^+$, $CD25^+$ lymphocytes caused by the retrovirus HTLV-I.[45] No chemotherapeutic regimen appeared successful in altering the survival of these patients, who have a median survival duration of only 9 months. The retrovirus, HTLV-I, encodes a transactivating protein tax that indirectly stimulates the transcription of numerous host genes including those of IL-2 and IL2R∀. The malignant ATL cells constitutively express approximately 10,000 IL-2R∀ subunits identified by the anti-Tac monoclonal antibody, whereas most of the patients' normal resting cells do not express this receptor. These observations stimulated us to perform therapeutic trials with the unmodified murine version of the anti-Tac monoclonal antibody. Six of the 19 patients treated developed a partial (4) or complete (2) remission, in one case still persisting over

12 years after initiation of therapy.[45] Using the murine model of human ATL we developed, we demonstrated that the effector action of anti-Tac appears, in part, to be due to a form of ADCC involving Fc(R III receptors[46] In patients in the early phase of their ATL the effective action of anit-Tac involves both ADCC and the interruption of the interaction of IL-2 with its growth factor receptor which, in turn, leads to cytokine deprivation mediated apoptotic cell death.

Despite the encouraging results in these studies, there were three problems inherent in the use of the murine anti-Tac monoclonal antibody that included its immunogenicity, its ineffectiveness in recruiting host-effector functions and its short in vivo survival. To address these issues, I joined with Cary Queen, then of the Biochemistry Branch of the National Cancer Institute but subsequently of Protein Design Labs, to humanize anti-Tac.[51] Humanized anti-Tac retains the complementary determining regions (cdr) from the mouse whereas virtually all of the remainder of the molecule is derived from human IgG1k. The humanized version of anti-Tac is virtually nonimmunogenic, has improved pharmacokinetics (T ½ survival of 40 hr for the murine as compared to 20 days for the humanized version) and functions in antibody-dependent cellular cytotoxicity with human mononuclear cells in contrast to the absence of this effector function with its parent murine version.[51] After our encouranging phase 1/II trials Hoffmann-LaRoche, Inc. conducted two double-blind, placebo controlled randomized trials involving 535 evaluated patients to determine the value of humanized anti-Tac (daclizumab) in preventing renal allograft rejection. In each trial, the patients received a standard immunosuppressive regimen. The parallel treatment groups also received either intravenous placebo or daclizumab prior to the transplant and on four subsequent

occasions. No drug specific adverse events or increased morbidity were observed. Acute rejection episodes were reduced by 40% in the patients treated with daclizumab ($p<0.01$); 98% of the patients receiving triple immunosuppression and daclizumab retained their renal allograft for at least 6 months whereas only 92% of the patients in the placebo controlled group retained their grafts ($p=0.02$).[52] As noted above, on the basis of these phase III clinical trials, daclizumab has received approval for use in the prevention of acute renal rejection episodes in patients undergoing kidney transplantation. In addition to its use in the prevention of organ allograft rejection our collaborators have shown that humanized anti-Tac is of value in the therapy of T-cell mediated autoimmune disorders including uveitis, multiple sclerosis, and tropical spastic paraparesis. For example, in a clinical trial, patients with noninfectious uveitis who were receiving multiple immunosuppressive agents were weaned off their systemic immunosuppressive medications and in parallel received daclizumab infusions every 4 weeks.[53] In 9 of 10 patients who were treated with daclizumab over a 4-year period, improvement was noted in visual acuity without the use of the previously required immunosuppressive agents. On the basis of these encouraging findings a phase III controlled trial is being initiated involving humanized anti-Tac therapy for patients with active noninfectious uveitis.

Systemic Radioimmunotherapy Directed to IL-2R∀

One limitation in the use of unmodified monoclonal antibodies in the treatment of leukemia and lymphoma is that they are relatively ineffective as cytocidal agents. This is especially true with late stage HTLV-I associated ATL, wherein the leukemic cells continue to express IL-2R∀ but no longer produce nor require IL-2 for their proliferation. This limited efficacy of unmodifife monoclonal antibodies in cancer therapy has led to

an alternative approach that involves the use of agents such as anti-Tac as carriers of cytotoxic substances including toxins or radionuclides. In one series of studies, we have collaborated with Ira Pastan and Robert Kreitman of the NCI in the evaluation of their IL-2R∀ directed immunotoxin, LMB-2, that includes a truncated version of *pseudomonas exotoxin* A linked with anti-Tac fv to yield a single antibody toxin fusion protein that is used in the therapy of patients with IL-2R∀ expressing leukemias and lymphomas. Our dominant efforts however have been directed toward the development of a generalized reproducible approach for the systemic radioimmunotherapy of IL-2R∀ expressing malignancy. We augmented the efficacy of murine and humanized anti-Tac by arming the monoclonal antibody with the ∃-emitting radionuclide ^{90}Y, and observed a partial or complete remission in over 50% of patients with ATL treated in clinical trials.(47) In sum the progressive increase in our understanding of the IL-2 receptor and its involvement in disease has opened the possibility for more specific immune intervention. The clinical application of IL-2R receptor directed therapy has represented a novel approach for the treatment of certain neoplastic diseases, select autoimmune disorders, and for the prevention of organ allograft rejection.

Future Therapeutic Directions: IL-15 as a Therapeutic Agent and IL-2/15R$ Directed Therapy for Autoimmune Diseases

In the future in our translational clinical trials program we wish to exploit our expanding understanding of the IL-15/IL-15 receptor system in the normal immune response and its disorders in disease. The opposing effects of IL-2 and IL-15 discussed above have implications for the use of these cytokines as elements in cancer therapy strategies and as components of vaccines. IL-2 has been approved for use in metastatic renal carcinoma and malignant melanoma; however, in the presence of IL-2, the tumor-

specific T-cells generated may interpret the tumor cells as self and by the activation induced cell-death (AICD) process may undergo apoptotic cell death. Furthermore, the inhibition mediated by IL-2 on the survival of those memory $CD8^+$ T-cells that are directed toward cancer-associated antigens is not desirable. In contrast, IL-15 with its activation of T-cells, its inhibitory action on AICD and its facilitation of the persistence of memory phenotype $CD8^+$ T-cells may be superior to IL-2 in the treatment of cancer and as a component of vaccines. Thus in our future plans we wish to evaluate IL-15 as a therapeutic agent. We are encouraged by the observations of Yutaka Tagaya that syngeneic tumor cells injected intravenously into IL-15 transgenic mice did not develop metastatic foci in the lungs whereas in wild type mice pulmonary tumor masses developed within 2 to 3 weeks. Following preclinical evaluation of IL-15 in murine tumor models, we hope to evaluate IL-15 as a replacement for IL-2 therapy of malignant melanoma and renal cell carcinomas and as a component of vaccines for cancer and AIDS.

Our previous studies using intact unmodified monoclonal antibodies have targeted IL-2R∀ the private receptor for IL-2. Although such IL-2R∀ directed therapy has met with considerable success which culminated in the approval by the FDA of the humanized version of the anti-Tac antibody (daclizumab), such approaches directed toward this subunit have their limitations. In particular, antibodies directed to IL-2R∀ do not inhibit the actions of IL-15, a cytokine that does not bind to this receptor subunit. An antibody, humanized Mik∃1 (HuMik∃1) that acts on the IL-2/IL-15R∃ receptor subunit shared by IL-2 and IL-15 blocks all actions of IL-15 but not those of IL-2 on T and NK cells. We have shown that the administration of HuMik∃1 as a single agent leads to

prolonged cardiac allograft survival in cynomolgus monkeys. We plan to initiate clinical trials with this agent in patients with diseases where a disorder of IL-15 has been observed, and where this abnormality of the inflammatory cytokine IL-15 has been proposed to play a role in the disease pathogenesis. In particular, we plan clinical trials of HuMik∃1 in groups of patients with T-cell large granular lymphocytic leukemia associated with hematocytopenia, tropical spastic paraparesis, rheumatoid arthritis and multiple sclerosis. These studies may help us test the hypothesis that IL-15 plays a pathogenic role in these disorders and to determine whether disrupting IL-15 interaction with its beta receptor subunit will provide clinical benefit to patients with such disorders.

As I look to the future, the task of preventing and curing cancer is a difficult one. The road ahead seems long and daunting. I am, however, encouraged about cancer research in general and in the use of immune approaches in particular, in preventing, diagnosing, and treating cancer. Recent advances in the knowledge of disordered cytokine and growth factor receptor expression by neoplastic cells, taken in conjunction with progress in efforts directed toward the linkage of toxins and radionuclides to monoclonal antibodies or their fragments, and in the genetic engineering of these antibodies to produce humanized versions of the monoclonal antibodies with reduced immunogenicity, improved pharmacokinetics, and functions provide new hope for the treatment of neoplastic disease. We have come close to fulfilling the vision of Paul Ehrlich, who in his Croonian lecture entitled, On Immunity with Special Reference to Cell Life, 100 years ago, stated:

"It is hoped that immunizations such as these which are of great theoretic interest may also come to be available for clinical application…attacking epithelial new formations,

particularly carcinoma, by means of specific anti-epithelial sera…I trust my lords and gentlemen that we no longer find ourselves lost on a boundless sea but that we have already caught a distinct glimpse of the land where we hope, nay, which we expect, will yield rich treasures for biology and therapeutics."

It must be emphasized that the outcome of the efforts to prevent and cure cancer is not solely in the hand of scientists. Cancer research including that involving immunological approaches is a public endeavor requiring the support of all of our society. What we have is the hope that all of us together will ennoble humankind by seeing this great and hopeful adventure through to its end.

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