

## Career centers on study of immune system

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Until the year that I was a sophomore in college, when serious infections occurred in humans, they generally were attributed to unusually virulent infectious agents thought to be present in the community. No one had considered that there could be a heightened susceptibility to infection for the person in whom the infection occurred.

As with many great discoveries, the first example of a human host defect was found serendipitously in 1952 when a young boy with repeated infections was presented to a consultant who suggested that, with all of the many infections he had had, he surely must have a very high level of gamma globulin. Applying the then new technology of serum electrophoresis, the boy was found to have just the opposite, i.e, a near absence of gamma globulins.

That observation opened the door to numerous remarkable and insightful discoveries of inborn errors of the immune system over the ensuing 52 years. Each of them led to further elucidation of immune system functions. It has been my privilege to be a participatory observer of these discoveries during most of this half century, a period that many consider the most exciting period in the history of immunology.

In the 10-year period after the identification of Bruton's agammaglobulinemia, several other immune defects were recognized on the basis of their consistent clinical findings or inheritance patterns, including the Wiskott-Aldrich syndrome, 'Swiss type' agammaglobulinemia, and dysgammaglobulinemia with elevated 19S gamma-globulins but a deficiency of 7S gamma-globulins, now called the Hyper IgM syndrome. One of the most puzzling conditions was described by Charles Janeway, Sr., MD, and his colleagues in patients who had recurrent pyogenic infections but, unlike those with Bruton's disease, also had enlarged lymph nodes, hepatosplenomegaly and hypergammaglobulinemia. That condition later proved to be X-linked chronic granulomatous disease.

As a post-doctoral fellow in allergy and immunology in the early 1960's, I became very interested in immune system functions because of frequent parental reports of recurrent infections in the young patients we cared for. Because of Bruton's report, serum protein electrophoresis was performed frequently on the sera of such patients, however, very few other tests were available to evaluate immune function at that time.

From 1961-1972, the role of the thymus gland in immune system function began to be elucidated, and the tissues of origin for antibody-producing cells were found. These discoveries came about through a combination of studies in experimental animals and of insight provided by another human "experiment of nature." Angelo DiGeorge, MD, had observed that infants who died with congenital hypoparathyroidism had no thymic tissue at autopsy and that the cause of death was opportunistic infection. Studies *in vivo* showed cellular immunity to be impaired in such patients, but they were not deficient in gamma globulins. These aggregate findings and observations led to the concept of division of immune system functions into two compartments, with Bruton's agammaglobulinemia being an example of a "pure" antibody deficiency and DiGeorge's syndrome an example of a "pure" cellular immunodeficiency.

In 1966, another important chance discovery was made by Beulah Holmes, Paul Quie, and co-workers, who were testing the capacity of antibiotics to penetrate cell walls. They found that bacteria ingested by phagocytic cells from patients with fatal granulomatous disease were protected from killing by antibiotics in the culture medium but, unlike the situation with normal donors' cells, were also not killed within the patients' phagocytic cells. Metabolic studies of these patients' cells showed lack of a respiratory burst upon activation of the hexose monophosphate shunt pathway during phagocytosis. This provided the first insight into the later discovery of several defects of oxidative metabolism in these patients.

In 1968, as a junior faculty member, I received a consultation request to evaluate a 12-year-old boy admitted to the surgical service for removal of an aspergillus lung abscess. Physical examination revealed multiple scars from incisions and drainages of more than 200 abscesses over his young lifetime. Being aware of Holmes' observations, tests of neutrophil oxidative metabolism and bacterial killing after phagocytosis were performed to determine if the patient had chronic granulomatous disease; all of these studies were normal. However, an intradermal skin test with *Candida albicans* extract was performed to elicit a delayed hypersensitivity response as part of the evaluation of his cellular immune

function and, paradoxically, there was an enormous immediate wheal and flare reaction.

The discovery of IgE as the immunoglobulin fraction bearing reaginic antibody activity had just been reported in 1967, so I wondered if this child could have an excessive amount of IgE, even though he had no history to suggest atopy. That proved to be the case, as his serum IgE level was greater than 32,000 international units/ml.

Another young boy with recurrent subcutaneous abscesses and staphylococcal pneumonias was referred to me shortly after that and, like the first, he also had a markedly elevated serum IgE level. These first two patients with the Hyper IgE syndrome were remarkably similar to each other and to the more than 40 other patients I have seen with this condition over the past three decades. Other unique features include the pronounced tendency of these patients to develop giant persistent pneumatoceles after staphylococcal pneumonia, a history of multiple bony fractures due to osteopenia, and coarse facial features. Eosinophilia of blood, sputum and tissues, including the walls of excised lung cysts, is a consistent finding. Others have reported that they also have delayed shedding of their primary teeth. Despite many efforts by myself and by numerous other investigators to discover the fundamental host defect leading to the formation of recurrent cutaneous, pulmonary and visceral abscesses and to identify the mutated gene responsible for the Hyper IgE syndrome, neither have been accomplished as of yet.

A series of technological advances in the 1960s and 1970s helped the investigation of immune system function enormously. Soon after the identification of immunoglobulin classes and subclasses, largely through the study of human myeloma proteins, antisera were produced that could be used in immunoelectrophoresis studies or in single radial diffusion assays to quantify individual immunoglobulin concentrations. This led to the discovery of IgA deficiency by two immunologists who were testing each other with these technologies. However, it was not until the 1970s that methods were found to distinguish between T and B lymphocytes. In 1970, Pernis first reported that rabbit B cells bore surface immunoglobulin. Soon after that, several workers reported the serendipitous observation that sheep erythrocytes bound to a subset of human lymphocytes, forming a "rosette;" this subset ultimately proved to include both T and natural killer (NK) cells. Thus, for the first time, there were methods available to count both T and B cells in the circulation. This capability improved dramatically with the advent of monoclonal antibodies and flow cytometry in the late 1970s.

The past 50 years have seen enormous progress in this field. An unknown concept until 1952, there are now more than 120 different primary immunodeficiency syndromes in the world's literature. Until the past decade, there was little insight into the fundamental problems underlying a majority of these conditions. It is impressive that the underlying genetic defects have now been identified in more than 50. Advances in the treatment of these diseases have also been remarkable. Antibody replacement has been improved greatly by the development of human immunoglobulin preparations that can be safely administered by the intravenous route, and cytokine and humanized anti-cytokine therapies are now possible through recombinant technologies.

For most of the past three decades, my research has focused on infants with severe combined immunodeficiency (SCID). SCID is a syndrome of diverse genetic origin characterized by profound deficiencies of T and B cell and, in some cases, NK cell function. Affected infants present in the first few months of life with diarrhea and failure to thrive. Persistent infections with opportunistic organisms, such as *Candida albicans*, *Pneumocystis carinii*, varicella, adenovirus, respiratory syncytial virus, parainfluenza 3, cytomegalovirus, Epstein-Barr virus (EBV), and bacillus Calmette-Guerin (BCG), lead to death. These infants also lack the ability to reject allografts, leaving them at risk for fatal graft-versus-host disease (GVHD). SCID is uniformly fatal in the first two years of life unless immune reconstitution can be accomplished.

Since infants with SCID have no T cells (which constitute 70% of normal lymphocytes), they are lymphopenic; recognition of this characteristic alone can result in early diagnosis—even at birth. Their lymphocytes fail to proliferate *in vitro* in response to mitogens, antigens or allogeneic cells. Serum immunoglobulins and antibodies are diminished to absent. The thymuses are very small (usually less than 1g) and lack thymocytes, corticomedullary distinction and Hassall's corpuscles. However, recent studies from our group have shown that these thymuses are capable of supporting T cell development when normal stem cells are provided. Thymus-dependent areas of the spleen are devoid of lymphocytes, and lymph nodes and tonsils are absent.

Flow cytometric studies have shown that there are unique lymphocyte phenotypes for the various genetic forms of SCID, with some having B cells but no T or NK cells (so-called T<sup>-</sup>B<sup>+</sup>NK<sup>-</sup> SCID), others having no T cells but normal or elevated

numbers of B and NK cells (T<sup>+</sup>B<sup>+</sup>NK<sup>+</sup> SCID), others having no T or B cells but many NK cells (T<sup>-</sup>B<sup>-</sup>NK<sup>+</sup> SCID), and others having extremely low numbers of all types of lymphocytes (T<sup>-</sup>B<sup>-</sup>NK<sup>-</sup> SCID).

After the first report of human SCID more than 5 decades ago, it became clear—as different patterns of inheritance were appreciated—that there was more than one cause for this condition. In some families there was clearly X-linked recessive inheritance, whereas in others there was autosomal recessive inheritance. The first discovered molecular cause of human SCID, adenosine deaminase deficiency, was reported in 1972. However, no further causes of this condition were identified until 1993 when the molecular basis of X-linked SCID was discovered.

Over the past 11 years, remarkable progress has been made in elucidating several other causes of this syndrome. Advances in molecular biology and the human genome project, and increased knowledge of various components of the immune system through studies of mutant mice and humans with genetically-determined immuno-deficiencies have all contributed to this understanding. It is now known that SCID can be caused in humans by mutations in at least 10 different genes, and the likelihood is that there are other causes yet to be discovered.

The gene products of 3 of the mutated genes are components of cytokine receptors (the IL-2 receptor  $\gamma$  chain that is also shared with 5 other cytokine receptors (IL-4R, IL-7R, IL-9R, IL-15R and IL-21R), Jak3 – the primary signal transducer from the common  $\gamma$  chain, and the  $\alpha$  chain of the IL-7 receptor); whereas the products of 5 more genes are necessary for antigen receptor development (RAG1, RAG2, Artemis, CD3 $\delta$  and CD3 $\epsilon$ ); the product of one gene (adenosine deaminase) is necessary for detoxification of metabolic products of the purine salvage pathway that cause lymphocytes to apoptose; and the final gene encodes CD45, a phosphatase that serves as a critical regulator of signaling thresholds in immune cells. The most common form of SCID is the X-linked type, caused by mutations in *IL-2RG*, accounting for 46% of cases at my institution. This is followed by adenosine deaminase deficiency in 16% of cases, and IL-7R $\alpha$ -chain deficiency in 11% of cases.

SCID is a pediatric emergency. Life-saving immune reconstitution can be achieved in patients with SCID by administering either HLA-identical sibling marrow or rigorously T cell-depleted related haploidentical bone marrow stem cells. Since these infants lack T cells, they are unable to reject a graft and therefore do not need to receive toxic chemotherapy prior to transplantation. The ability to use T cell-depleted parental marrow has been the most important therapeutic advance for infants with SCID in the past two decades. It has made immune reconstitution and survival possible for virtually all such infants if diagnosed before untreatable infections develop. Taking the T cells out also avoids having to use immunosuppressive drugs after the transplant to prevent graft-versus-host disease; this allows immune function to come in, the ultimate goal.

Nearly all cases could be diagnosed at birth if routine blood counts and manual differentials were done and flow cytometry and T cell functional studies performed when lymphocyte counts are below the newborn normal range (2,000-11,000/mm<sup>3</sup>). Effective treatment could then be given shortly after birth, with an expected survival rate of more than 95%. During the past 22.5 years, my colleagues and I have transplanted 38 SCID infants within the first 3.5 months of their lives and 37, or 97%, survive.

Although bone marrow transplantation has been life-saving for these infants, however, it is not a perfect therapy with many such infants having remaining B cell defects. The hope has been that gene therapy could be used to effect immune reconstitution in this condition. ADA deficiency was the first genetic defect in which gene therapy was attempted; those early efforts were unsuccessful. However, within the past 4 years, a normal cDNA was successfully transduced into autologous marrow cells of nine infants with X-linked SCID by retroviral gene transfer by the group in France headed by Alain Fischer, MD, with subsequent full correction of their T cell defects and without the need for IVIG therapy. This offered hope that gene therapy would eventually be the treatment of choice for all patients with SCID or other genetically-determined immunodeficiency diseases for whom the molecular basis is known. Unfortunately, two of these children developed leukemia-like clonal proliferations of T cells, so gene therapy trials were halted with the hope that this problem can be overcome.

Having been an allergy/immunology Training Program Director for nearly 30 years, I enjoyed being a mentor and teacher for the post-doctoral fellows who came into our program and have followed my trainees' accomplishments with pride. I have been a member then fellow of the AAAAI for more than 40 years. I have had the honor and privilege of serving as

President, of receiving a Distinguished Service Award and of receiving an Honorary Fellow Award from this organization. Throughout this time I have enjoyed many collegial interactions with its members and fellows. I was recently elected to the Institute of Medicine of the National Academies of Science.