MEDICAL GRAND ROUNDS

"THE MOLECULAR BASIS OF X-LINKED HUMAN PRIMARY IMMUNODEFICIENCY DISEASE"

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INTRODUCTION

In the last year, the field of immunology has witnessed an extraordinary explosion of information in several areas making it difficult to focus on a single discovery of major clinical significance. However, among these many exciting developments the characterization of the precise molecular defects for three primary human immunodeficiency diseases has provided a quantum leap in our understanding of normal lymphocyte physiology as well as suggested important avenues for possible therapeutic interventions.

Today I will discuss with you in broad general terms the concepts of primary versus secondary immunodeficiency, review the various types of primary immunodeficiency diseases, and discuss in some detail the primary immunodeficiency diseases, especially those in which the molecular defects have recently been elucidated. I will very briefly touch on other single gene defects among this group of disorders where significant therapeutic advances have been made.

Primary Immunodeficiency Diseases

Primary immunodeficiencies are distinguished from secondary immunodeficiencies based on their hereditary nature. The primary immunodeficiency diseases are a heterogenous group of over 50 disorders which affect the cells and proteins of the immune system. While these disorders were originally thought to be rare and to occur only in infants and young children, clinical experience has taught us that they are rather common in older children, adolescents, adults and often result in mild clinical disease. While relatively uncommon, I remind you that some of these diseases are more common than cystic fibrosis,

the most common genetic abnormalty in Caucasians.

In general terms, the primary immunodeficiency diseases can be divided into those that affect the four major components of host defense: the B lymphocyte system, the T lymphocyte system, the phagocytic system, and the complement system (Table I). Each of these systems plays a critical role in some phase of the immune response and each system has its own set of immunodeficiency problems, and in particular, clinical syndromes associated with it. However, these syndromes are most easily understood from the perspective of infectious diseases. That is, the infecting microorganisms correlate with particular components of the immune system and in many respects represent the earliest indication to the physician that problems exist. For example, children that have repeated bacterial infections with pneumococci are most likely to have difficulties in their B lymphocyte or antibody producing system whereas children with chronic granulomatous disease more typically have infections with Staph aureus, Salmonella, Serratia, Klebsiella, Pseudomonas and the like (Clinical Immunology Spectrum, 1993).

While immunodeficiency diseases are characterized by undue susceptibility to infection, paradoxically, many are also characterized by autoimmune diseases and/or excessive production of IgE antibodies. Due to the ability of modern antibiotics to control many types of infections, autoimmune diseases now account for increasing morbidity among immunodeficiency patients. Finally, there is an increased incidence of malignancy in patients with immunodeficiency diseases. Whether this is due to defective tumor immunosurveilance is unknown (Stiehm, 1992).

TABLE I

SOME PRIMARY IMMUNODEFICIENCY DISEASES

B-LYMPHOCYTE DEFECTS	T-LYMPHOCYTE DEFECTS
X-linked Agammaglobulinemia (XLA)	Purine Nucleoside Phosphorylase Deficiency
Hyper IgM Syndrome (HIGM)	Chronic Mucocutaneous Candidiasis
Selective IgA Deficiency	DiGeorge Anomaly

COMBINED B- AND T-LYMPHOCYTE DEFECTS
Severe Combined Immunodeficiency (SCID)
Adenosine Deaminase Deficiency (ADA)
Wiskott-Aldrich Syndrome
Ataxia Telangiectasia

PHAGOCYTIC CELL DEFECTS	COMPLEMENT DEFICIENCIES
Chronic Granulomatous Disease	C1 Deficiency
Leukocyte Adhesion Defect	C4 Deficiency
Neutropenia	C2 Deficiency
Chediak Higashi Syndrome	C5 Deficiency

As mentioned above, with the exception of selective IgA deficiency (which is present in about 1 and 500 births) genetically determined immunodeficiency is rare. B cell defects far out number those affecting T cells, phagocytic cells or complement components. Although general population statistics are difficult to obtain, X-linked agammaglobulinemia (XLA) occurs with a frequency of 1 in 50,000 and severe combined immunodeficiency (SCID) in about 1 in 100,000. During childhood, there is a 5 to 1 male to female sex preponderance for these disorders. This reverses into adulthood where males and females are affected about evenly.

Genetics of Immunodeficiency Disorders

The faulty genes for many immunodeficiencies are known to be on the X chromosome. They have been localized to specific sites of the X chromosome in the case of X-linked agammaglobulinemia, X-linked severe combined immunodeficiency, the Wiskott-Aldrich Syndrome, X-linked lymphoproliferative disease, X-linked hyper IgM syndrome, and X-linked chronic granulomatous disease (Table II).

Normal Human B Lymphocyte Physiology

Prior to a detailed discussion of immunodeficiency, I would like to spend a few moments discussing our current notions of B cell physiology. B lymphocytes mature within the bone marrow and leave the marrow expressing a unique antigen binding receptor, the immunoglobulin molecule. Each B lymphocyte has a different receptor. This membrane bound glycoprotein has been the subject of many of my previous Medical Grand Rounds. The membrane receptors for antigen on B and T cells are illustrated in Figure 1 below. The B cells have about 10^5 molecules of membrane bound antibody receptor per cell. Each antibody molecule possesses identical specificity for antigen and can interact directly with antigen. T lymphocytes have about the same number of receptors called "T cell receptors", each with identical specificities. T cells bearing CD4 recognize antigen associated with class II MHC molecules and generally function as T helper cells. T cells bearing CD8 recognize antigen associated with class I MHC and generally function as T cytotoxic cells.

One of the major developments in the field of B cell physiology over the last few years is the understanding that accessory molecules that have been long known to exist on T cells exist on B cells as well. Several such molecules are responsible for not only holding the B cell receptor on the plasma membrane, but participate in the signalling process. It is one of these key molecules that is defective in X-linked agammaglobulinemia (Smith, et al., 1993).

TABLE II

FEATURES OF SOME HUMAN PRIMARY IMMUNODEFICIENCY DISORDERS INVOLVING LYMPHOCYTES

DISORDER	FUNCTIONAL DEFICIENCIES	PRESUMED CELLULAR LEVEL OF DEFECT	PRESUMED MOLECULAR LEVEL OF DEFECT
X-linked agammaglobulinemia	Antibody	Pre-B cell	Xq22; B cell-specific tyrosine kinase (src gene)
Immunodeficiency with elevated IgM	IgG, IgA, IgE antibodies	IgG, IgA, IgE, B lymphocytes; "switch" T cells	Xq26-27 (Ligand for CD40)
Common variable immunodeficiency	Antibody	B lymphocyte	Unknown
Selective IgA deficiency	IgA antibody	IgA B lymphocyte	?6p21.3
IgG subclass deficiency; kappa chain deficiency	Antibody	B lymphocyte; immuno-globulin heavy or light chain gene deletions (rarely)	2p11; 14q32.3
X-linked lymphoproliferative disease	IgG subclasses; anti-EBNA antibody	B cell; ?also T cell	Xq25-26
DiGeorge Anomalie	T cellular; some antibody	Dysmorphogenesis of 3rd and 4th branchial pouches	Unknown
Severe combined immunodeficiency syndromes (autosomal recessive; ADA deficiency; X-linked recessive;)	Antibody and T cellular;	Unknown; metabolic defect(s); ?T cell; ?stem cell; ?recombinase deficiency	Xq13.1-21.1 (γ chain IL2R)
Nezelof's syndrome (including with PNP deficiency	T cellular, some antibody	Unknown; ?thymus; ?T cell; metabolic defects	14q3.1 (PNP deficiency)
Cartilage-hair hypoplasia	T cellular	G1 cycle of many cells	RFX (?) CIITA (?)
Wiskott-Aldrich syndrome	Antibody, T cellular	Unknown	Xp11-11.3
Ataxia telangiectasia	Antibody, T cellular	B lymphocyte; helper T lymphocyte	11q22.3
MHC antibody deficiencies	Antibody, T cellular	Defective synthesis of Class I or II molecules	Unknown
Leukocyte adhesion deficiencies (LAD types 1 & 2)	Cytotoxic lymphocytes, phagocytic cells	All leukocytes	21q22.3; 95 Kd MW beta chain (CD18) of CD11a (LFA-1), CD11b (CR3) and CD11c (p150,95); Siayl-Lewis X ligand of E selectin
Hyperimmunoglobulinemia E	Specific immune responses; excessive IgE	Unknown	Unknown

Adapted from Buckley, 1993

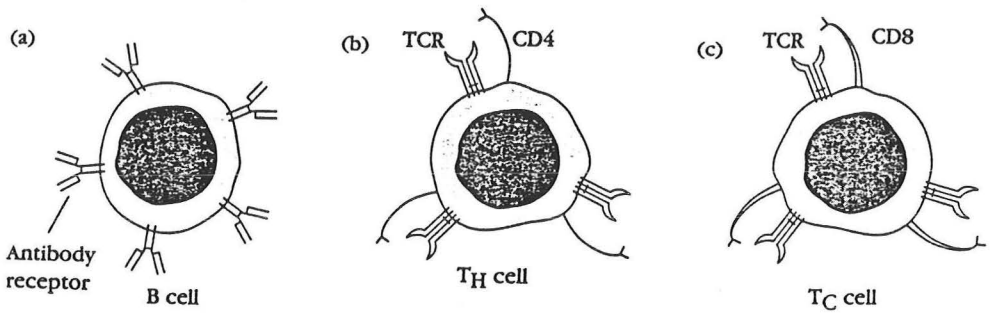

Figure 1. The membrane receptors for antigen on B and T cells.

Let us stop for a moment and discuss how an immature B cell becomes an antibody producing plasma cell. In general, the process is thought to take place in two separate ways. First, a maturational process that is antigen independent. Second, a clonal selection process that is antigen dependent. As is illustrated in Figure 2, immature B cells differentiate and put immunoglobulin on their surface. However, it is only after B cell activation occurs with the introduction of antigen that B cells proliferate, further differentiate and in two separate pathways lead to memory B cells and plasma cells which make specific antibody. Plasma cells are fully differentiated B cells with highly efficient secretory capabilities. The difference between the primary and secondary immune response is a dramatic increase in the amount of serum antibody during a secondary immune response (Figure 3). I illustrate this primarily for the B lymphocyte side and antibodies although a similar phenomenon occurs on the T cell side. This secondary immune response is built into separate components. First, there is "more" antibody present in a secondary immune response than in a primary response and second, the antibody is "better" in a secondary response than a primary response. That is, the antibody has a higher affinity for

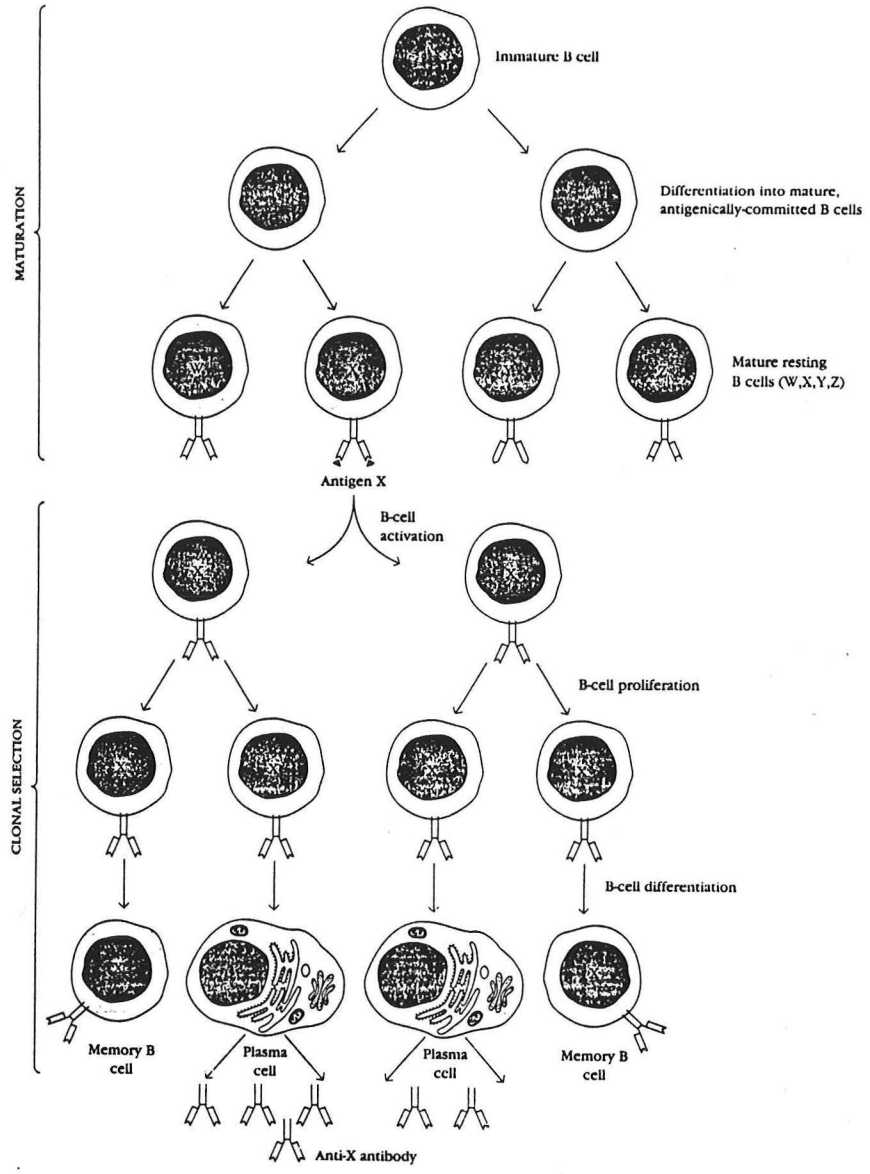

Figure 2. Maturation and clonal selection of B lymphocytes.

antigen. These higher affinity circulating antibodies are, in many respects, the result of two independent processes. The first is the process of somatic mutation in which there is variation in the sequence around the hypervariable regions such that the fit between antigen and antibody is enhanced. The second process that occurs during a secondary immune response is the so-called "class switch". That is, IgM producing cells switch to produce IgG, IgA and IgE antibodies of the same specificity. This process is at the core of one the primary immunodeficiency syndromes. You

can imagine that this class switching phenomenon is a highly regulated event and we will see that this switching process is defective in the Hyper IgM Syndrome.

In addition to these processes, the cellular interactions involved in the production of antibody are legion. Again, I will focus on the B cell. You will recall that T helper cells are essential in order to trigger B cells to make significant amounts of antibody and these helper cells secrete a number of lymphokines, for example, IL-2, IL-4, IL-5, IL-6, IL-10 and interferon gamma. These cytokines are critical for the differentiation of a B cell into plasma cells and B memory cells.

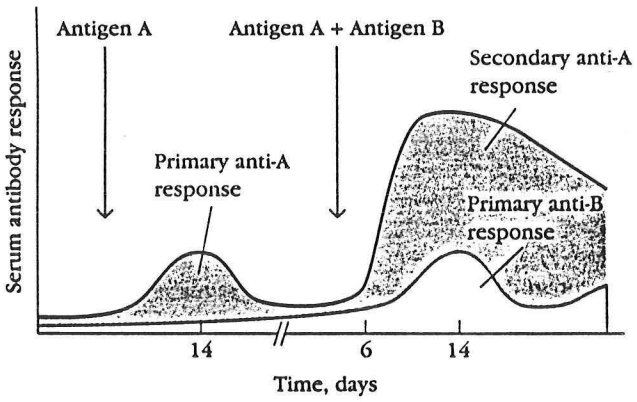

Figure 3. Differences in primary and secondary response to antigen.

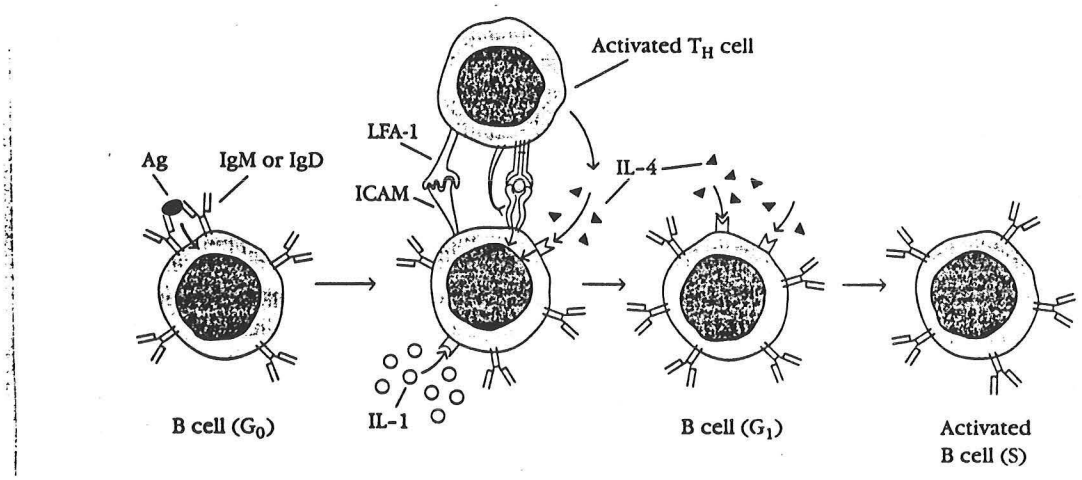

Figure 4. Generation of the humoral response begins with antigen cross linkage of the B cell membrane antibody receptor. The T_H cell/B cell interaction is much more complex (see Fig. 6).

B lymphocyte Defects

The B lymphocyte defects that I would like to speak about today are X-linked agammaglobulinemia (XLA or Bruton's agammaglobulinemia) and the hyperIgM syndrome.

The interplay between clinic and laboratory has set the stage for a contemporary analysis of primary and secondary immunodeficiency diseases of man, and in particular, for an analysis of the major perturbations of the immunologic system as they occur. Immunologists know that the concepts of B and T cells serving separate and distinct functions emerged from of the primary immunodeficiencies decades ago. These defects have, from the beginning of our field, been instrumental in allowing immunologists to understand more and more of normal lymphocyte physiology. Indeed as I hope to point out today, this is continuing as two novel molecules uncovered by careful study of human diseases have been described that undoubtedly play a critical role in normal B cell physiology. The physician-scientists who were instrumental in much of this work are among the leaders in the field of immunology (Robert Good, Fred Rosen, Max Cooper, Paul Kincade, Henry Kunkel).

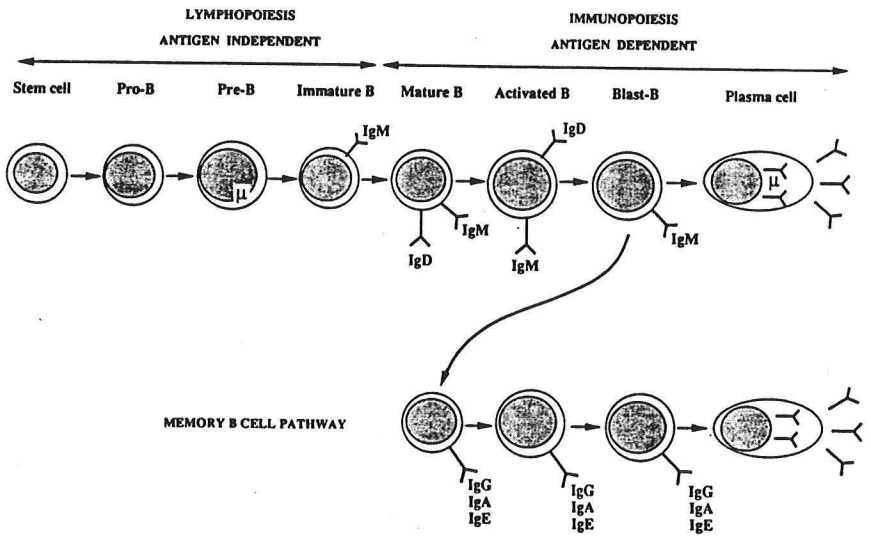

Figure 5. Simplest representation of human B lymphocyte differentiation (from Banchereau and Rousset, 1992).

It was from their work and from these rare examples of human disease that the first notions that B cells made antibody and T cells were involved in cell mediated immunity emerged. The role of the thymus, the bone marrow and primary and secondary lymphoid organs became known and even the distinction between primary and secondary immunodeficiency became clear. These investigators noted early that patients with different immunologic deficiencies developed entirely different syndromes of infection and laid the ground work for our understanding of the role of the various arms of the immune system in protection against and recovery from infectious disease. Thus, much of what we understand today about the immune system in infection largely derives from fundamental work done in the primary immunodeficiency syndromes. Additionally, the first human bone marrow transplantations performed between unrelated donors were done among patients with immunodeficiency disease more than 20 years ago. Even the involvement of the immune system in tumor surveillance was first appreciated over 2 decades ago in studies of patients with primary immunodeficiency disease.

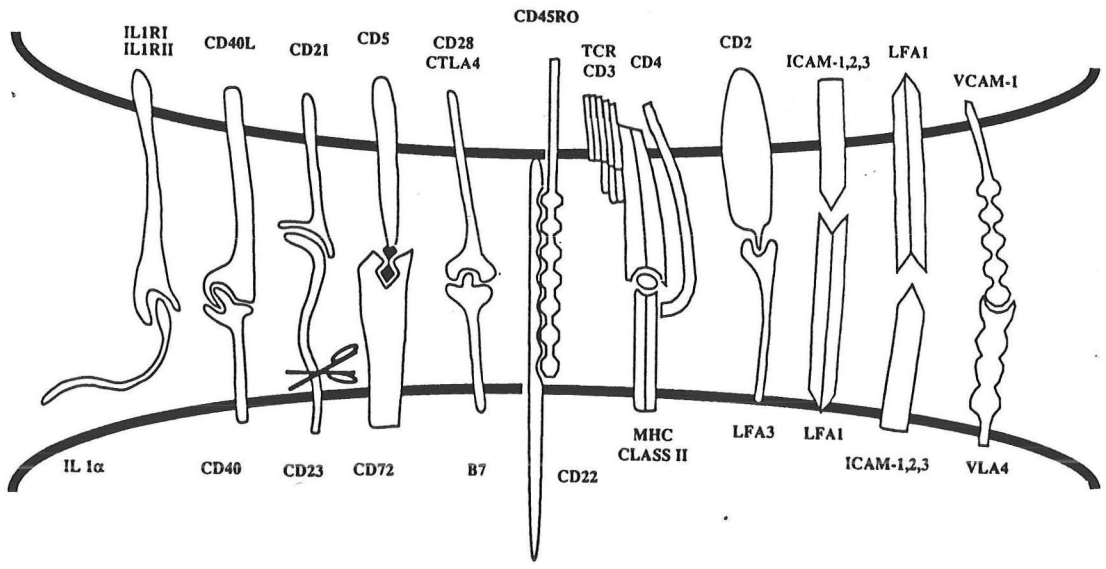

Figure 6. Schematic representation of the molecular interactons between B cells and help CD4+ T cells. Especially note the CD40L/CD40 interaction (from Banchereau and Rousset, 1992).

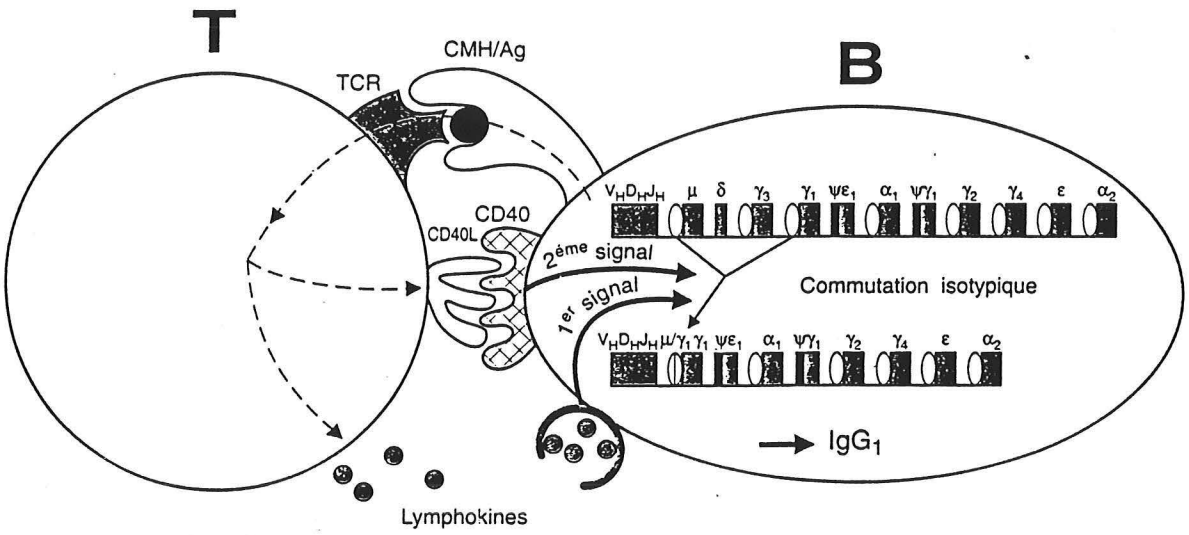

Figure 7. Simple scheme of isotype switching.

X-LINKED AGAMMAGLOBULINEMIA (XLA) (BRUTON'S AGAMMAGLOBULINEMIA)

The first described immunodeficiency disease in man was Bruton's agammaglobulinemia described by Ogden Bruton in 1952. His original case report follows.

> A boy, age 8 years, was well until age 4 years, when pneumonia with rubeola developed. The birth, developmental history, and family history were normal; a male sibling was well. At age 4 1/2 years, the patient developed chills, fever, and pain in the left knee. Physical examination was unremarkable except for a few petechiae on the arms and tenderness of the knee. The white blood cell (WBC) count was 16,400/mm^3 with 88 percent neutrophils; a blood culture was negative. The pain and fever disappeared with penicillin. Two weeks later, fever recurred; pneumonia was diagnosed and sulfadiazine was given. Four days later, mumps and gastroenteritis developed and the boy was admitted to the hospital. He experienced a prolonged febrile course, complicated by recurrent otitis. The throat, blood, and ear cultures were positive for Type XIV pneumococcus. After being treated with penicillin for 10 days, he improved. Otitis media developed 2 months later. Two days after antibiotics were discontinued for an ear infection, fever and left shoulder pain developed. The WBC count was 25,000/mm^3 with 91 percent neutrophils. Antibiotics were again begun. Six months later, recurrent fever occurred and Type XXXIII pneumococcus was recovered from the blood stream.
>
> After recover, a tonsillectomy and adenoidectomy were performed in the hope of decreasing the number of infections. However, in the next 4 years, the patient experienced 15 episodes of high fever due to sepsis; on seven occasions, pneumococci were cultured from the blood. He also had two episodes of pneumonia, three episodes of otitis, and two episodes of mumps. Prophylactic sulfadiznine and pneumococcal vaccine were ineffective in controlling the infections (Bruton, 1952).

I have personally followed about a dozen such boys. I would like to report to you an adult with this disorder who has been followed at our medical center by Dr. Richard Wasserman.

> A man presented to Aston Center in June 1987 at age 34. He had been diagnosed with Bruton's X-linked Agammaglobulinemia by Dr. Robert Good in the early 1960's after experiencing chronic sinopulmonary infections and polio which left him with a mild left lower extremity paresis. He was treated initially with intramuscular gammaglobulin on a somewhat irregular schedule and over the next twenty years was followed by various physicians. In the early 1980's he was treated with multiple courses of fresh frozen plasma in an effort to provide better gammaglobulin supplementation. That exposure resulted in non-A, non-B hepatitis which lead to chronic active hepatitis

by the time he was first seen in 1987.

At that time, he had no detectable IgA or IgM and IgG was supplied exogenously. His treatment regimen was modified to increase his doses of intravenous gammaglobulin and he was started on home administration of gammaglobulin. He experienced significant improvement of his sinopulmonary infections with a markedly decreased need for treatment antibiotics. Ascites and peripheral edema as well as malnutrition and wasting were, however, major problems. He was admitted in the summer of 1988 because of increased ascites, weight loss and fever. Echovirus 11 was grown from CSF and stool, although he did not have features of Chronic Enteroviral Meningoencephalitis Syndrome seen in agammaglobulinemic patients. Because of progressive malnutrition and wasting secondary to liver disease, he underwent orthotopic liver transplant in November 1988 and did well. Despite treatment with a lot of gammaglobulin specifically selected for high titers against Echovirus 11 at very high doses, Echovirus was cultured from his transplanted liver within two weeks. He has, however, continued to do well on cyclosporin and prednisone with hypertension as his only major complication from his liver transplant. The combination of high dose gammaglobulin and orthotopic liver transplant have transformed this patient's life. He has resumed working full time and is very active, hiking and camping in the New Mexico mountains for recreation. He experiences sinopulmonary infections requiring antibiotic therapy about once or twice a year. He has not been hospitalized for infection in more than 5 years. In 1992, he had an episode of Lyme Disease diagnosed on the basis of erythema chronicum migrans and responded well to a single course of antibiotics.

Both of these cases illustrate the cardinal features of X-linked agammaglobulinemia (XLA). Affected males are usually well for the first few months of life and then begin to develop frequent infections particularly of the paranasal sinuses and the lungs. Individual infections may be no more severe than in the general population but they may be chronic or recurrent. The most frequently identified pathogens are *S. pneumoniae, H. influenza, streptococci* and *staphylococci*, nontypeable *H. influenza*, moraxella, a variety of gram negative bacilli and mycoplasma may also become important pathogens in these patients with chronic lung disease. Bacterial meningitis, sepsis, arthritis and osteomyelitis occurs much less often but with substantially higher frequency than in normal populations.

The diagnosis of XLA is usually suggested by frequent and/or severe bacterial infections. When present, a positive family history of previously affected males is helpful in suggesting the presence of immunodeficiency. However, a positive family history is not always present since

not all cases have previously affected family members. Regular infusions of intravenous gammaglobulin are of real benefit in this disorder and prevent and/or delay the development of chronic lung disease.

The molecular defect in X-linked agammaglobulinemia has been sought for 40 years. It was initially thought that the antibody genes would be defective in such patients. However, this has not been found to be correct. Affected males lack circulating mature B cells and serum immunoglobulins of all isotypes are absent. Affected males have a normal number of pre-B cells in their bone marrow suggesting that the XLA defect resides in the pathway of B cell development. The defect is specific to the B cell lineage because other lymphocyte populations are normal. Heterozygous females appear immunologically normal as a result of selection against B cells having the mutant X chromosome active. Such "skewing" of X chromosome activity in the B cell population reliability identifies the carrier status. Recently, two groups, one headed by David Bently at Guy's and Thomas's Hospitals in the United Kingdom and Owen Witte at UCLA have defined the molecular basis of the defect in XLA. Each used a different approach to isolate what represents a novel protein tyrosine kinase. These represent the first mutations in *src* related genes that are involved in human genetic disease.

As an aside and to indicate the extraordinary competition in this area, both of these papers were published in January, 1993, the Bentley paper in *Nature* (Vetrie et al., 1993), the Witte paper in *Cell* (Tsukada, et al., 1993). The Witte paper was received on December 18, accepted and revised on December 31. Few of us can anticipate review, revision and acceptance in 13 days!

The Guy's hospital group used a fairly novel cloning strategy that employed yeast

artificial chromosomes. It had long been known that the gene for XLA resided on the X chromosome and the specific region of the X chromosome Xq21.3-Xq22. The consensus order of the loci in this region was established with various polymorphic markers showing no recombination with disease. Long range restriction maps had been constructed over this area with yeast artificial chromosomes and the identification of CPG islands in the region around DXS178 indicated the presence of genes in this area. 6.5 megabases of DNA were overlapped with 68 YAC clones forming the basis for a systematic search for the gene involved in XLA. Subtractive cDNA libraries, PCR amplification, southern blots, expression of genomic material and detection of DNA rearrangements in XLA families focused the attention of this group on a single cDNA. The availability of several patients with this disorder was crucial in the eventual identification of the disease. Figure 8 below illustrates some of the mapping strategies and the identification of the DNA rearrangements in XLA families.

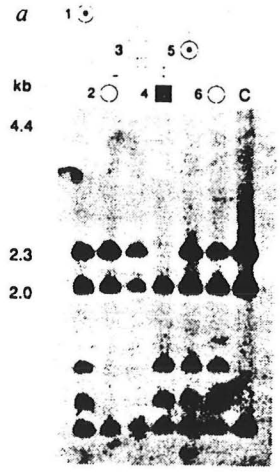

Figure 8. Identification of restriction fragment alterations in XLA patients (Vetrie et al., 1993).

The Bently group named the gene *atk* for agammaglobulinemia tyrosine kinase. (The UCLA group called it BPK for B cell Progenitor Kinase.) The gene encodes a polypeptide chain of approximately 500 amino acids. The sequence is most similar to that of the *src* family

of cellular oncogenes (Cantley et al., 1991). Members of the *src* gene family are characterized by extensive homology to the kinase domain of the transforming gene of the Rous sarcoma virus and encode intracellular nonreceptor protein tyrosine kinases. These proteins are usually bound to the membrane by myristylation and/or direct attachment to membrane proteins. It is thought that members of the src family play important roles in the cellular signaling pathways that regulate cell proliferation and differentiation. Protein tyrosine kinases function as central switching agents in these pathways by catalyzing the phosphorylation of tyrosine residues in specific target proteins.

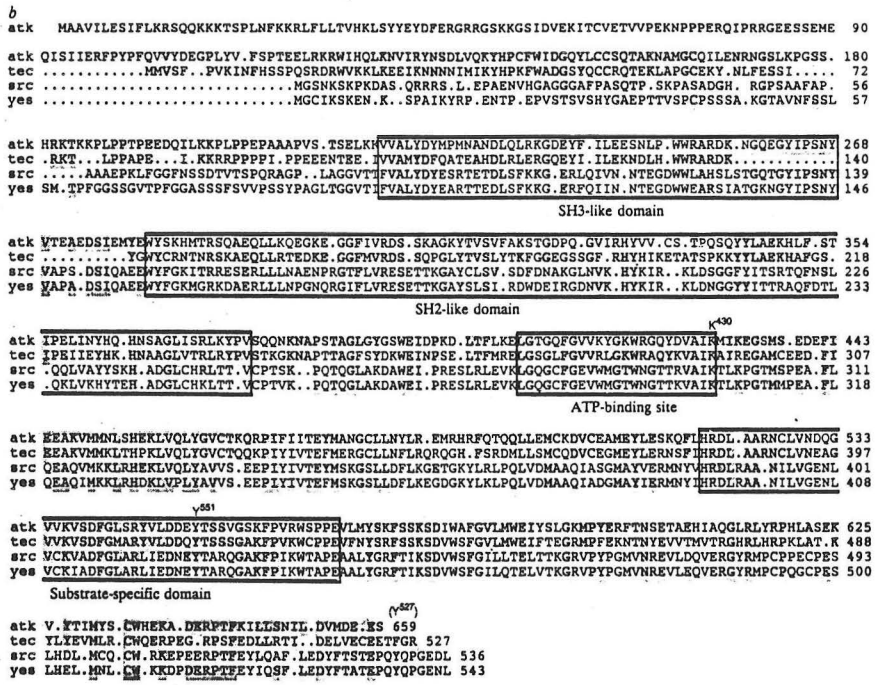

Figure 9. Comparisons of the predicted amino acid sequence of atk with that of closely related proteins of the src family (Vetrie et al., 1993).

The open reading frame of the sequence shows a high degree of similarity with two members of this gene family, the murine *tec* gene and the drosophila *src* 28C gene. The domain contains the ATP binding site. It also possess a putative kinase domain of 250 amino acid

residues which shows 64% amino acid sequence identity to the *tec* protein. The domain contains the ATP binding site including the conserved glycine at position 295 which is present in all functional protein tyrosine kinases. This is illustrated in Figure 9.

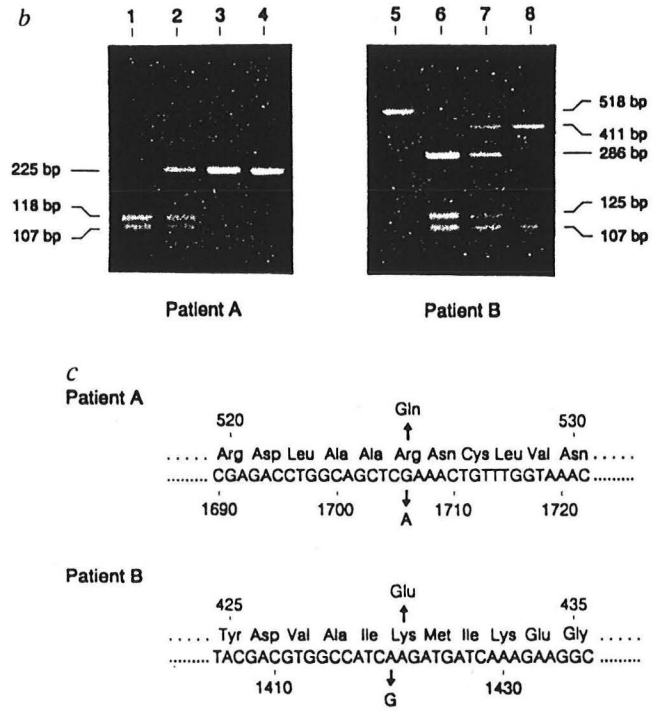

Figure 10. Diagram of part of the cDNA encompassing the segment of atk that was amplified in various patients (Vetrie et al., 1993).

The authors then examined the kinase domain of the *atk* gene in a number of patients using the polymerase chain reaction. Direct DNA sequencing of the relevant amplified products in two patients characterized a different point mutation in each. Patient A had a G to A transition at position 1706 whereas patient B had an A to G transition at 1420 (Fig. 10). Both of these would encode amino acid interchanges and both are nonconserved amino acid substitutions. They would likely have highly determinal affects on the catalytic function of the putative protein kinase. These are also illustrated in figure 11. Both point mutations would be expected to eliminate kinase activity of the proposed *atk* protein product.

By appropriate analysis, the authors were able to demonstrate that the particular gene that they cloned maps to the Xq21.3-Xq22 region of the X chromosome. They demonstrated

that the *atk* cDNA identified abnormalities in 8 different XLA patients. The knowledge that the gene encodes a novel tyrosine kinase which is expressed in B cells but not T cells is consist-

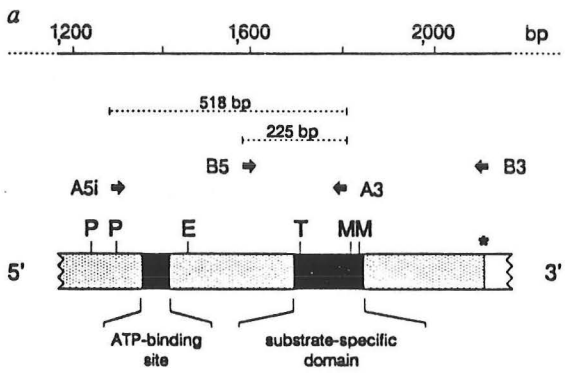

Figure 11. Model of the atk gene.

ent with all the information that has been gathered on X-linked agammaglobulinemia. Thus, the failure of pre-B cells to develop into mature circulating B cells could be the result of the *atk* gene product failing to fulfill its role in B cell signaling. In heterozygous females, pre-B cells whose active X chromosome expresses the mutant gene would not terminally differentiate and proliferate. This would explain why XLA carrier females show skewed X chromosome activity in their B cell lineage.

While the number of genes that encode intracellular protein tyrosine kinases in the human genome is large, only a few have been isolated and the protein products of even fewer have been analyzed. *atk* is the first member of the human src family of oncogenes which encode intracellular protein tyrosine kinases to be directly implicated in causing a human genetic disease. It is useful to reflect back on some brilliant mouse models of immunodeficiency that have been recently developed that involve so called "knockout" or homologous recombination. Three src family members of tyrosine kinases: *src, fyn*, and *lck* have been inactivated by

homologous recombination. The *lck* mutation results in a thymocyte differentiation defect and the other two result in immunodeficiencies in the B cell lineage. It will probably be of considerable interest to see if *atk* is involved in human neoplastic disease specific to the B cell lineage.

Witte's group approached the problem in a slightly different way and isolated the same gene. They mainly focused on cell lineage specific expression of transcripts and demonstrated that the molecule they refer to as BPK for B cell Progenitor Kinase had the same characteristics of *atk* described by Bentley's group. Specifically, it mapped to the same region, it was a novel cytoplasmic tyrosine kinase, it was expressed only in B cells, it mapped to the long arm of the X chromosome by flourescent in situ hybridization (FISH), it was tightly linked to the region of Xq22 identified as the XLA locus and most importantly, they demonstrated deficient expression of this gene in the EBV transformed B cell lines from patients with X-Linked Agammaglobulinemia. This latter is illustrated in Figure 12.

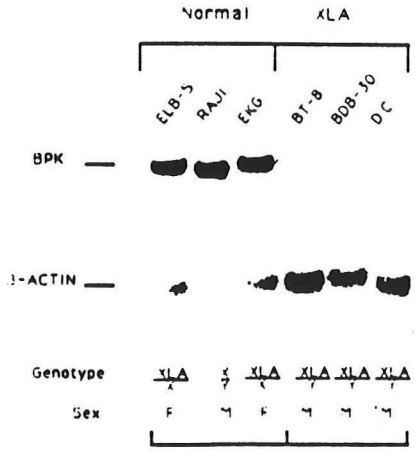

Figure 12. An absence or reduced expression of BPK mRNA in EBV transformed B cell lines from patients with XLA (Tsukada et al., 1993).

I have learned from several of my colleagues that over 25 other patients with this disorder have been identified and the mutant gene sequenced. Essentially every mutation is unique.

HYPER-IGM SYNDROME AND THE CD40 LIGAND

As described above, It has long been known that immunoglobulin production was essentially a two-step process. In early B cells, VDJ rearrangement takes place and surface IgM and IgG are displayed as the antigen specific receptors on B cells. These low affinity molecules presumably serve in the early immune response but for *bona fide* humoral immunity, two critical events need to take place: somatic mutation and class switching. These two processes are intimately related and are essential for the full development of the immunological repertoire.

While both of these processes take place in B cells, T cell "help" is essential and the abnormalities in antibody production in individuals with various forms of T cell deficiency are known to all of you. How T cells "help" antibody producing cells has long been a mystery. However, several of the critical factors that are involved in this process have been elucidated within the last few years and it is in this arena that CD40, the CD40 ligand and the hyper-IgM syndrome come together.

In 1984, Paulie et al., produced a monoclonal antibody to a human bladder carcinoma antigen and later showed that the molecule was also present on human B lymphocytes (Paulie, et al., 1985). This molecule (p50) was later designated CD40 and in 1986 Clark and Ledbetter showed that CD40 was critical in B cell activation.

In 1989, Stamenkovic et al. cloned the cDNA for CD40 and noted a relationship between the structure of this molecule and nerve growth factor receptor. They showed that the mRNA for CD40 was induced by gamma interferon in B cells. Next, antibodies to CD40 were produced and several investigators, particularly Jacques Banchereau, demonstrated convincingly that

antibodies to CD40 could be used for the longterm growth of human B lymphocytes, a subject that I discussed at my Medical Grand Rounds 18 months ago. Utilizing antibodies to crosslink CD40 turned out to be crucial in understanding normal B cell physiology. CD40 is now known to be a cell surface protein expressed on B lymphocytes, dendritic cells, follicular dendritic cells, normal epithelial cells and some epithelium carcinomas. When the TNF receptor family of proteins became elucidated, it was clear that CD40 was a member of this family. This family includes such molecules as the low affinity receptor for nerve growth factor, both forms of TNF receptors, CD27, OX40 and the lymphoma marker CD30. Monoclonal antibodies directed against CD40 had profound effects, including short and longterm proliferation (profound cytokines and present), differentiation, induction of intercellular adhesion and tyrosine phosphorylation of a series of intracellular proteins (Banchereau et al., 1991; Rousset et al., 1991, 1992). Taken together, these data strongly suggested that CD40 was the receptor for an unknown ligand.

The murine CD40 ligand (also known as TRAP or TNF-Related Activation Protein) was eventually isolated by two different laboratories (Armitage et al., 1993; Spriggs et al., 1992). Interestingly, the human CD40 ligand had also been isolated as a cDNA expressed in activated human T cells whose function was not known (Graf, et al., 1992). However, the authors noted its homology to TNF. The CD40 ligand is a 261 amino acid membrane spanning protein. Northern blot and FACs analysis showed that the human CD40 ligand is restricted in its expression to T lymphocytes; in particular, the CD40+ T cell subpopulation known to be associated with helper activity. Cells transfected with the human CD40 ligand cause the proliferation of human tonsilar B cells in the absence of costimuli and in the presence of IL-4, induces immunoglobulin secretion from purified B cells. Thus, both the receptor and the ligand

critical for B cell development were cloned, sequenced and expressed. Subsequently, several experiments documented the role of these molecules, and in addition, the additional role of a number of cytokines such IL-2, IL-4, and IL-10 and TGFβ in the production of immunoglobulins, and in particular, in class switching by human B cells. When the CD40 ligand was mapped to the X chromosome (specifically Xq26.3-27.1) the stage was set for analysis of the CD40 ligand in human immunodeficiency syndromes.

Immunodeficiency with hyperimmunoglobulin-M (hyper-IgM) is a syndrome characterized by low or absent IgG and IgA in serum and normal to elevated serum levels of IgM. Clinically, patients with this syndrome are similar to patients with other humoral immunodeficiencies, with frequent infections and an increased risk of neoplasm at an early age. The vast majority of patients with this order have a pattern of X-linked inheritance although there are similar syndromes without this genetics. Despite the heterogeneity of backgrounds, a common thread has been the inability of cultured B cells from these patients to switch from secreting IgM to secreting IgG or IgA. Several studies had been done to demonstrate that the pathogenic event in most of these patients involved their inability to switch from IgM to IgG *in vitro* and *in vivo*. **Indeed, the B cells from most patients with Hyper IgM syndrome could be induced to switch with T cells from normal individuals.**

> A 5 month old boy presented to another hospital with a several day history of fever and cough. Physical examination showed a normally grown infant with tachypnea, rales and retractions. Chest roentgenogram revealed diffuse interstitial infiltrates suggestive of *Pneumocystis* carinii pneumonia. He responded well to treatment with intravenous trimethoprim-sulfa and glucocorticoids. A maternal uncle had died of *Pneumocystis* pneumonia at age 15 with a life long history of recurrent infection, cyclic neutropenia, rheumatoid arthritis and obliterative intrahepatic cholangitis. Immunologic evaluation was markedly abnormal:
>
> IgA <7 mg/dl
> IgG <40 mg/dl
> IgM 65 mg/dl
> Absolute lymphocyte count 5200

CD3+ T cells 55%
CD4+ T cells 38%
B cells 41% (3xnormal)
Normal response to mitogen stimulation with phytohemagglutinin

A tentative diagnosis of hyper-IgM syndrome was made but severe Common Variable Immunodeficiency could not be excluded. (The elevated B cells ruled out X-linked Agammaglobulinemia.)

Intravenous gammaglobulin was administered and following a 14 day treatment course of high dose trimethoprim-sulfa, pneumocystis prophylaxis was begun with the same drug. The patient's family moved to the Metroplex when he was 18 months old. Gammaglobulin treatment and antibiotic prophylaxis was continued and he did well. In the Spring of 1993 peripheral blood was assayed for the expression of CD40 ligand by flow cytometry and no CD40 ligand was detected.

The Mutations of CD40 Ligand in Hyper-IgM

A crucial lead was the mapping of the gene for the CD40 ligand to the long arm of the X chromosome (Xq26.3-27.1) (Graf et al., 1992). Also known to map in this region (Xq24-27) was the unidentified gene responsible for HIGM. B cells produce normal amounts of IgM appropriately in response to antigenic challenge, but fail to switch to produce IgG, IgA and IgE and to establish memory. T-cell dysfunction was suspected in the disease because, unlike other antibody-deficient patients, some HIGM patients are surprisingly prone to certain infections commonly associated T-cell deficiency, such as *Pneumocystis carinii* pneumonia and cryptosporidial diarrhea. T cells were further implicated by an elegant study which demonstrated that HIGM B cells could be made to produce normal amounts of IgG, IgA and IgE if co-cultured with a malignant human T-cell line (Mayer et al., 1986).

Table IV. Serum immunoglobulin levels and B-cell phenotype.

Subject	Serum immunoglobulin mg/dl			%CD19$^+$ cells	% CD19$^+$ cells with surface(s) immunoglobulin staining			
	IgM	IgG	IgA		sIgM	sIgD	sIgG	sIgA
Patient								
1	82	20	<7	27	99	99	<1	<1
2	672	20	<7	8	99	99	<1	<1
3	89	50	<7	13	99	99	<1	<1
Control (n=3)	50-200	>600	50-200	12-200	60-80	60-70	10-20	10-20

Serum immunoglobulin levels were determined using a radial immunodiffusion technique. Peripheral blood mononuclear cells (PBMC) were stain for B cell number using a phycoerythrin-labeled anti-CD19 mAb (Becton Dickenson), and surface immunoglobulin staining was assessed by fluorescein-labeled $F(ab')_2$ fragments of goat anti-human immunoglobulin (Boehringer Mannheim). CD19$^+$, CD19-positive.

With this background, five groups set out to investigate the role of the CD40 ligand in HIGM. They looked at a total of 18 unrelated individuals with HIGM. Activated T cells from 17 of these individuals failed to bind an engineered soluble form of CD40; in the eighteenth, only weak binding was seen (Korthäuer, et al., 1993; Ramesh et al., 1993; Fulehan et al., 1993; DiSanto et al., 1993; Allen et al., 1993; and Aruffo et al., 1993). **The CD40 ligand genes from 13 patients were then sequenced and 12 different point mutations or deletions were discovered.** DiSanto, *et al.* show that, in three of their cases, the defective gene is carried by the asymptomatic mother. Four of the groups showed that the functional defect in B cells seen in HIGM was due to failure to cross-link CD40. Cross-linking CD40 restores the ability of HIGM B cells to proliferate and to undergo isotype switching when co-cultured with appropriate cytokines (Hill & Chapel, 1993).

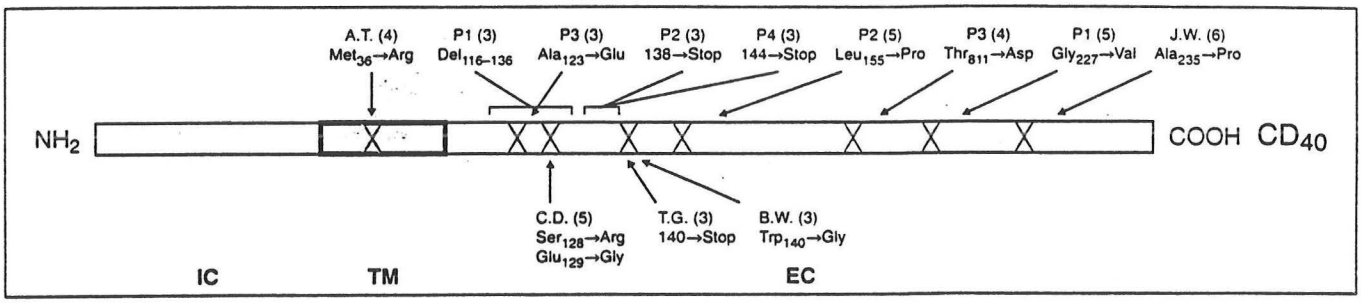

Figure 13. Schematic representation of the coding region of the CD40 ligand and the mutations reported in 12 Hyper-IgM patients.

Next it was shown that the defective CD40 ligand gene is indeed responsible for the T cells' inability to bind CD40. DiSanto *et al.* took advantage of a polymorphic microsatellite repeat in the 3' untranslated region of the CD40 ligand. The mother of one patient carried two allelic forms of this marker. Because X-chromosome inactivation is random in the HIGM1 carrier, only half of her activated T cells expressed functional CD40 ligand: all of the T cells that failed to bind soluble CD40 displayed the microsatellite marker associated with the defective CD40 ligand gene! Allen *et al.*, transfected cell lines with wildtype CD40 ligand or with identified HIGM mutant alleles. Whereas the wild-type transfectants bound CD40 and induced isotype switching in normal B cells, the lines transfected with the mutants failed to do so. Aruffo, *et al.*, reported similar results with soluble forms of the mutant CD40 ligand.

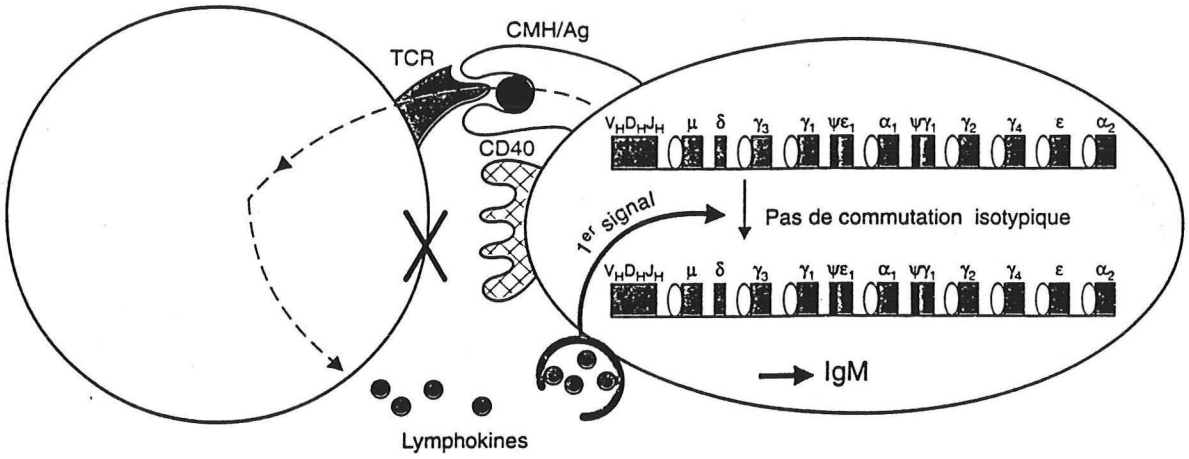

Figure 14. Schematic representation of the defect in CD40L/CD40 on the isotype switch.

Thus, knowledge of the pivotal importance of the CD40-CD40 ligand interaction in B-cell differentiation and isotype switching rapidly led to the identification of the CD40 ligand gene as responsible for HIGM. A closer study of the phenotype of the disease now provides further questions about the function *in vivo* of this ligand-receptor pair. There are two characteristic features HIGM which are easily explained by the currently identified role for the CD40 ligand in T-B cell cooperation. One is the mechanism by which inadequate T cell function fails to control intracellular parasites such as *Pneumocystis carinii*; further study of HIGM T cells may

help to shed light on this. It may be relevant that CD40 is expressed on epithelium, interdigitating cells and basophils as well as on B cells. To date, the CD40 ligand has been identified only on T cells (Ranheim & Kipps, 1993). The other, even more puzzling feature is the intermitant drop in granulocyte numbers which affects about half of the patients with this disease. This could be related to the presence of CD40 on some human hematopoietic progenitors.

For HIGM patients and clinical carriers detection will be an immediate benefit. Specific therapies, with genes and recombinant proteins are now possible although most patients remain well with adequate antibody replacement. The benefits of human gene therapy and the outcome of animal studies with the recombinant proteins are awaited.

In unpublished work that I'm aware of, at least three other groups have sequenced the CD40 ligand from an additional 11 patients with this disorder and in every instance there is a mutation in the CD40 ligand and in virtually every instance it is a unique mutation. Both missense frameshifts, deletions, altered reading frames, etc., have been described. All lead to the same general phenotype. These results document that defective expression of the CD40 ligand by T cells underlies the failure of isotype switching in the hyper-IgM syndrome. Obviously, these discoveries will make it extremely difficult to determine carrier identification in prenatal diagnosis as the mutation is different in every patient. However, with knowledge of the structure of these molecules such testing can now be envisioned. In addition, these discoveries open new avenues for therapy. It has been reported that Epstein Barr Virus transformed B cells transfected with human CD40L induced isotype switching to IgE in normal B cells treated with IL-4 (Spriggs et al., 1992; Faust et al., 1993). Experiments to demonstrate that expression of the normal CD40L gene in patient T cell lines correct their inability to induce

switch recombination in B cell. This will pave the way for future attempts at *in vivo* therapy (Armitage et al., 1993).

Table IV. IgE synthesis by PBMC and by B cell

		Patient			Control		
Cells	Stimulus	1	2	3	1	2	3
PBMC	-	<150	<150	<150	200	<150	<150
	IL-4	<150	<150	<150	3200	4700	5800
	CD40 mAb +IL-4	7100	2400	3700	7700	7600	9400
B cells	-	<150	<150	<150	<150	<150	<150
	IL-4	<150	<150	<150	<150	300	200
	CD40 mAb + IL-4	6600	5200	5400	10700	5320	7400

Net IgE synthesis (pg/ml) above background. Background IgE levels were detected in the presence of cycloheximide (100 μg/ml) and were always <500 pg/ml.

X-LINKED SEVERE COMBINED IMMUNODEFICIENCY

Severe combined immunodeficiency disease (XSCID) is characterized by severe and persistent infections from early life, which are due to profound impairment of both cellular and humoral immune function. SCID occurs in only one of every 10^6 live births. Classical XSCID is characterized by the absence of both T and B cells. It has long been presumed that the defect related to a lymphocytic stem cell. Autosomal recessive forms of SCID may also result from adenosine deaminase or purine nucleoside phosphorylase deficiencies, or can be associated with MHC complex class II deficiency such as the bare lymphocyte syndrome or IL-2 deficiencies. XSCID accounts from approximately half of SCID. The disease is characterized by an absence or diminished number of T cells and histologic evidence of hypoplasia and abnormal differentiation of the thymic epithelium. Levels of B cells can be normal or even elevated. The B cells, however, are not functional; therefore, males are hypo- or agammaglobulinemic. In some cases, the B cells can synthesize and secrete immunoglobulin when cultured with normal T cells. This suggests that a lack of T cell help could be the dominant problem rather than an intrinsic B cell defect. Indeed, after bone marrow transplantation, many patients develop post-transplantation antibody responsiveness.

I have spoken previously at these Grand Rounds about the critical roles that IL-2 and the IL-2 receptor play in the regulation of the immune response particularly in regulating the magnitude and duration of T cell responses following antigen activation. As you know, the IL-2 receptor is a target for many novel forms of immunotherapy in both autoimmune disease and malignancy. The IL-2 receptor (which was originally thought to be a single chain molecule) is now known to contain three chains, IL-2Rα, IL-2Rβ and IL-2Rγ. These three molecules result in the formation of three different classes of IL-2 receptors. Low affinity receptors contain IL-

2Rα but not IL-2Rβ or IL-2Rγ. Intermediate affinity IL-2 receptors contain IL-2Rβ and IL-2Rγ but not IL-2Rα and the high affinity receptors contain all three chains. The high and intermediate affinity receptors are important for IL-2 signaling. The molecular characterization of these three chains of the IL-2 receptor naturally lead to their chromosomal localization and IL-2Rγ was found to localize to the X chromosome at band Xq13, the same location as human X-linked severe combined immunodeficiency disease. Recently, Warren Leonard and his group have documented in three patients with XSCID that there is a distinct mutation of the IL-2Rγ gene and they propose that it is the genetic defect in this disease. XSCID which is characterized by the absence of T cells and severely diminished cell mediated and humoral immunity is the most recent human immunodeficiency disease to fall to molecular analysis. This work was published one month ago and has not been confirmed by other groups.

<u>IL-2Rγ is located on Xq13</u>.

Figure 15 shows the location of IL-2Rγ to Xq13 by in situ hybridization.

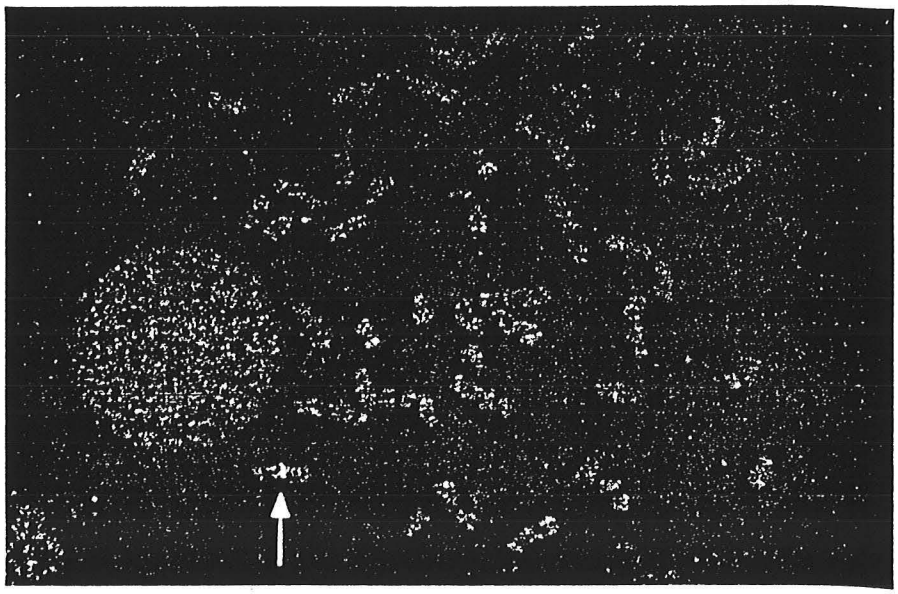

Figure 15. Localization of IL-2Rγ to Xq13 by in situ hybridization, Noguchi, et al., 1993.

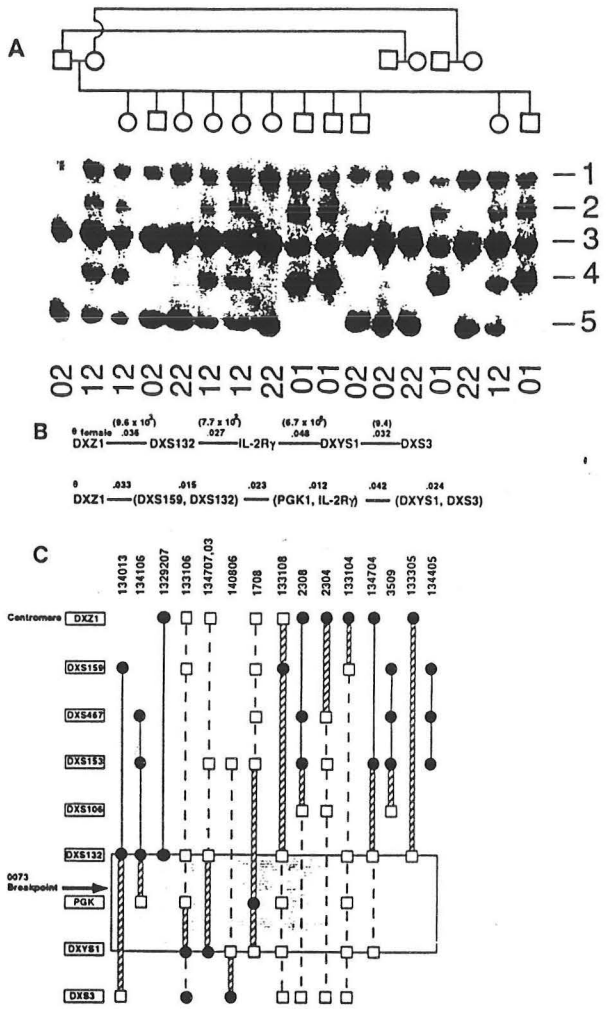

Figure 16. The IL-2Rγ gene is linked to the XSCID locus. Mendelian inheritance of IL-2Rγ gene in the three generation family. B) Recombinants between IL-2Rγ and other loci within the region Xcen-q22, Noguchi, et al., 1993.

Defects in each patient are illustrated in the B portion of the figure. Stop codons which are generated in patients two and three, result in a protein lacking the majority of the cytoplasmic domain including substantial parts of the *src* homology subdomain region contained in IL-2Rγ. In patient 1, 251 amino acids are deleted including the entire cytoplasmic domain. The genes were sequenced from several normals and found to be normal in each.

These data establish that XSCID in man is associated with mutation of the IL2-Rγ gene product.

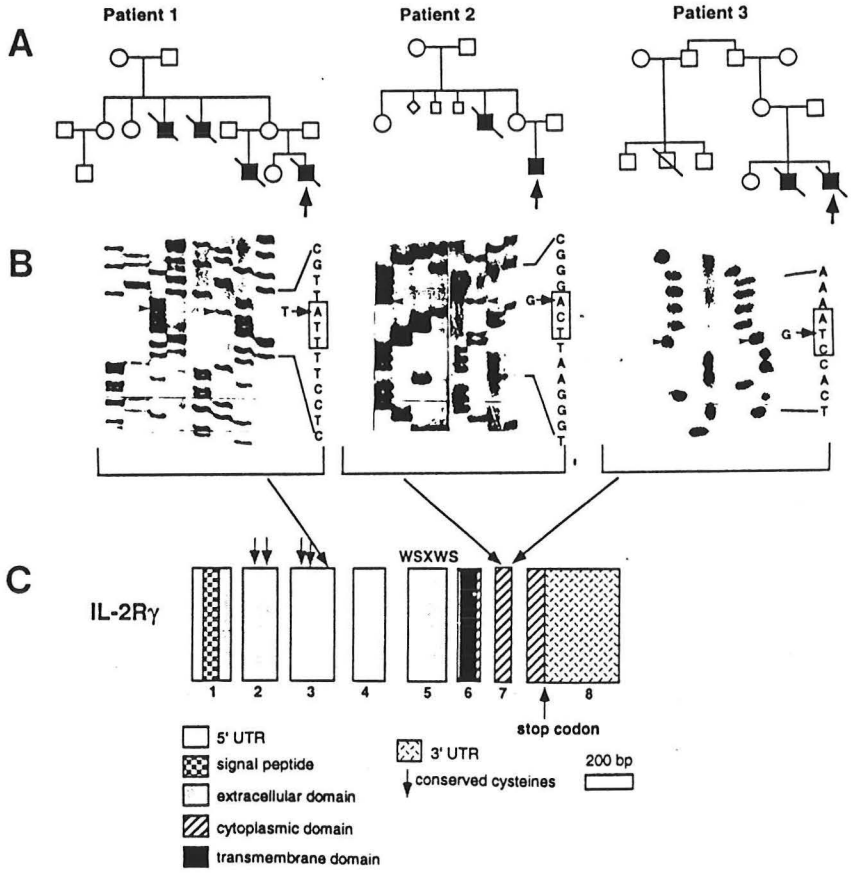

Figure 17. XSCID Patients have Mutations in the IL-2Rγ Gene. A) Pedigrees and histories of the XSCID patients studied. Circles, female; squares, male; closed squares, males with SCID; squares with slashes, deceased males. Small squares and diamond in the pedigree of patient 2 indicate miscarriages (male and of undetermined sex, respectively). B) Sequencing of XSCID IL-2Rγ gene sequences. Shown in the sequence of DNA from a normal donor (left panel in each pair) and of DNAs from patients 1, 2, and 3 (right panel in each pair). Patient 1 has an AAA (Lys) to TAA (stop codon) transversion in exon 3, resulting in a truncation of the carboxy-terminal 251 amino acids; patient 2 has a CGA (Arg) to TGA (stop codon) transition in exon 7, resulting in the truncation of 62 amino acids. The location of each mutation is indicated in the sequence to the right of each set of panels. The sequence shown is complementary to the coding strand. The boxed nucleotides for patients 1, 2, and 3 are complementary to TAA, TGA, and TAG stop codons, respectively. C) Schematic showing locations of the artificial stop codons (diagonal arrows) present in the XSCID patients. Patient 1 has a premature stop codon in exon 3, and patients 2 and 3 have premature stop codons in exon 7. TRR, untranslated region. (Noguchi, et al., 1993).

Implications

These findings suggest that IL2-Rγ plays a critical role in thymic maturation of bone marrow-derived precursor T cells. A number of reports have discussed the potential role of IL-2 receptors in thymocytes but the observation of these defects provides further evidence that thymic maturation in humans and mice may differ in their degree of dependence on the IL-2-IL-2 receptor system. Recall that mice with IL2 knockouts do not have profound immunodeficiency.

These findings suggest that mutation of other components of IL-2-IL-2 receptor system could be responsible autosomal recessive forms of SCID or less severe forms of immunodeficiency. In this regard, it is noteworthy that SCID has been associated with defective IL-2 production which in one case, at least, resulted from a defective production of NF-AT, a nuclear factor required for IL-2 gene transcription. However, the patient's cells were capable of producing low levels of IL-2 mRNA perhaps explaining why this SCID patient had T cells, and consistent with the possibility that the amount of IL-2 produced was sufficient for a degree of thymic maturation but not for normal mature T cell function in the periphery.

Other implications include: first, because XSCID individuals can recover B cell function following T cell engraftment, these findings suggest that the absence IL-2Rγ expression in B cells can be significantly compensated for by the presence of normal T cells during terminal B cell activation and differentiation.

Second, three distinct mutations were found in examining three patients. This suggests that there is no single dominant mutation as is found, for example, in cystic fibrosis. Although large numbers of XSCID patients need to be examined to determine the prevalence of each mutation, the fact that in 3 out of 3 cases the mutations were distinct, suggests that new mutations must constantly arise to replace the mutant X chromosome in males that are not passed on the next generation so that a variety of mutations would be expected. Finally, these findings have important implications for prenatal and post-natal diagnosis, carrier female identification and gene therapy for XSCID.

ADENOSINE DEAMINASE (ADA) DEFICIENCY

An absence of the enzyme ADA (encoded by a gene mapped to chromosome 20q.13-ter) has been observed in approximately 15-20 percent of patients with SCID (Hirschhorn, 1993). This has been shown to be due to a variety of point (Hirschhorn, et al., 1992; Markert, et al., 1989) and deletional mutations (Markert, et al., 1987) in the ADA gene. Marked accumulations of adenosine, 2'-deoxyadenosine and 2'-O-methyladenosine lead directly or indirectly to lymphocyte toxicity, which causes the immunodeficiency. Adenosine and deoxyadenosine are apparent suicide inactivators of the enzyme, S-adenosylhomocysteine (SAH) hydrolase, resulting in the accumulation of SAH. SAH is a potent inhibitor of virtually all cellular methylation reactions. Such patients usually have more profound lymphopenia than other SCID infants and rarely have elevated percentages of B cells. In further contrast to infants with other types of SCID, a few ADA deficient patients have been found to have rare Hassall's corpuscles in their thymuses and changes suggestive of early differentiation (Ratech, et al., 1989).

Other distinguishing features of ADA-deficient SCID's have included the presence of rib cage abnormalities similar to a rachitic rosary and multiple skeletal abnormalities of chondroosseous dysplasia on radiographic examination; these occur predominantly at the costochondral junctions, at the apophyses of the iliac bones and in the vertebral bodies (Chakravarti, et al., 1991). Enzyme replacement therapy with polyethylene glycol-modified bovine ADA (PEG-ADA) administered subcutaneously once weekly has resulted in both clinical and immunologic improvement in twenty-nine ADA-deficient patients (Hershfield, et al., 19987, 1993). However, this therapy should not be initiated if bone marrow transplantation is possible, as it will confer graft-rejection capability. This disease is a leading candidate for gene therapy and a form of gene therapy has already been attempted in mature T cells of at least two such

patients (Blaese, et al., 1993; Blaese, 1993). This was covered in last week's Medical Grand Rounds by Dr. Moreadith.

MOUSE MODELS OF IMMUNODEFICIENCY DISEASES

Approximately 3,500 genes of the mouse have been assigned to specific chromosomal locations. Many of these genes were first recognized by the occurrence of spontaneous mutations (Shultz and Sidman, 1987). Our understanding of mechanisms of heritable human immunological diseases has been advanced through the study of experimental animals bearing single gene mutations that perturb the immune system. Two immunological mutations interfere with the development of lymphoid organs; the nude (*nu*) mutation causes thymic aplasia while dominant hemimelia (*Dh*), causes splenic aplasia. Certain mutations such as *nu* and severe combined immunodeficiency (*scid*) are of great use as hosts for human and other xenogeneic tissues, (Schuler, 1990; Bosma & Carroll, 1991). While some mutations, such as beige (*bg*), are considered as homologues for specific human diseases, the major value of the immunological mutants are as tools with which to dissect the mammalian immune system (Quimby, et al., 1989; Rihova & Vetvicka, 1990).

The molecular basis of several of these mutations has recently been determined. For example, it has been shown that the steel (*Sl*) locus encodes a hematopoietic stem cell growth factor while the dominant spotting (*W*) locus encodes the *c-Kit* protooncogene, a tyrosine kinase receptor for this growth factor. Mutations at the *S1* or *W* loci cause severe abnormalities at the level of mast cells with accompanying deficiencies in pigment forming cells, red blood cells and germ cells (Shultz, 1991a). A mutation that causes osteopetrosis, a metabolic bone disorder characterized by an increase in skeletal mass, has also been found to be within a structural gene for a hematopoietic growth factor (Shultz, 1991b). Thus, mice homozygous for the osteopetrosis (*op)* mutation show extensive bone accumulation resulting from failure in the bone remodeling process. Such failure is secondary to defective osteoclast development accompanied by a

generalized macrophage developmental defect. Recently there is evidence that the *op* mutation is within the macrophage colony-stimulating factor (*C-sfm*) structural gene. Additionally, recent data have shown that the lymphoproliferation (*lpr*) mutation encodes a defective gene for the mouse Fas antigen (Cohen & Eiserberg, 1991). The failure of appropriate expression of this cell surface protein, that normally mediates apoptosis within the thymus, is thought to underlie the nonmalignant lymphadenopathy and systemic autoimmune disease characteristic of MRL/Mp-*lpr/lpr* mice (Theofilopoulous & Dixon, 1985; Theofilopoulous, et al., 1989) (Reviewed in Shultz, L.D., 1993).

I have recently learned that the murine XLA defect is in the same gene as the Bruton's defect I discussed earlier.

TABLE VII. Immunodeficiency Mutations of the Mouse

Gene Name	Gene Symbol	Chromosome	Major Phenotypic Characteristics
	Mutations that cause severe immunological defects		
Nude	*nu*	11	Congenital thymic aplasia; hairlessness Quantitative and functional T cell deficiency Increased NK cell and macrophage activity
Streaker	nu^{str}	11	Remutation at the nude locus in the AKR/J strain Similar to *nu* in phenotypic expression Homozygosity prevents T cell lymphoma development
Severe combined immunodeficiency	*scid*	16	Absence of functional T cells and B cells Lymphopenia, hypogammaglobulinemia Defect in rearrangement of genes that encode antigen-specific receptors on lymphocytes Normal hematopoietic microenvironment Normal antigen-presenting cell, myeloid and NK cell function DNA repair defect
Motheaten	*me*	6	Granulocytic skin lesions Impaired humoral and cell-mediated responses Decreased responses to T cell and B cell mitogens Deficient cytotoxic T cell and NK cell activity Short lifespan (3 weeks) associated with autoimmune pneumonitis
	Single gene models of less severe immunodeficiencies		
Dominant hemimelia	*Dh*	1	Defect in the splanchnic mesoderm in early embryos causes congenital absence of the spleen and widespread visceral and skeletal abnormalities Reduced serum IgM and IgG2 Impaired humoral antibody response Decreased numbers of lymph node mast cells
X-linked immunodeficiency	*xid*	X	B cell abnormalities result in defective humoral responses to type-II thymic-independent

			antigens and certain thymic-dependent antigens
Lipopoly-saccharide response	*Lps*	4	Resistance to lethal effects of endotoxin (LPS) Defective T cell and B cell response to Lipid A moiety of LPS Macrophage abnormalities include: resistance to lethal effects of LPS in vitro, reduced tumoricidal activity, and failure to respond to MIF
Hemolytic complement	*Hc*	2	Homozygotes (Hc^o/Hc^o) lack serum C5 Increased susceptibility to certain pathogens Impaired chemotactic responses of neutrophils Prolonged rejection of allografts
Beige	*bg*	13	Decreased endogenous NK cell activity Impaired cytotoxic T cell function Reduced granulocyte chemotaxis and cytotoxicity Lysosomal membrane defect
Dominant spotting	*W*	5	Encodes *c-kit*, protooncogene; receptor for SCF/KL Pleiotropic defects similar to those caused by mutations at the W locus Defective microenvironment
Hairless	*hr*	14	Decreased splenic T cell proliferative responses to alloantigens Reduced humoral responses to tetanus toxoid Increased incidence of thymic lymphoma Hairless by 5 weeks of age
Rhino	hr^{rh}	14	Reduced responses to thymic-dependent antigens Increased #'s Thy-1 pos epidermal dendritic cells Hairless by 5 weeks; skin becomes wrinkled and thickened
Immunodeficiency associated with systemic autoimmunity			
Lymphoproliferation	*lpr*	19	Mutation within the Fas gene Massive lymphadenopathy associated with proliferation of aberrant T cells Systemic autoimmunity
Generalized lymphoproliferative disease	*gld*	1	Lymphadenopathy and systemic autoimmunity similar to that in *lpr/lpr* mice May encode the ligand for the Fas antigen Aberrant monocytes in blood

Y-linked autoimmune accelerator	*Yaa*	Y	Systemic autoimmunity associated with B cell hyperplasia in susceptible strains
Immunodeficiency associated with primary endocrine defects			
Diabetes	*db*	4	Decreased cell-mediated immune function in vivo associated with hyperinsulinemia, obesity, and increased corticosterone levels
Obese	*ob*	6	Similar to *db*
Dwarf	*dw*	16	Transient loss in cortical thymocytes associated with primary defect in anterior pituitary

Adapted from Schultz, 1993

CONCLUSIONS

Within the past six months three astonishing discoveries have simultaneously unraveled the mystery of three different X-linked primary human immunodeficiency diseases. Once again, these discoveries emphasize that careful descriptions by clinicians combined with molecular biological approaches can forge new understandings of normal human physiology. Additionally, with bone marrow and stem cell transplants and indeed even retrovirally mediated gene transfer, Immunology, once again, is in the forefront of contemporary medical therapeutics.

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