Immunodeficiency Syndromes

This lecture traditionally goes beyond immunodeficiency and will also serve as a course review

Although a very large number of engineered defects in murine lymphocyte development have been generated in the last decade, naturally occurring disorders of lymphocyte development have (not too surprisingly!) been most thoroughly investigated in man. In this lecture the focus will primarily be on naturally occurring human immunodeficiencies that arise because of mutations in genes critical for lymphocyte development. We will examine the molecular mechanisms involved in inherited disorders of lymphocyte development in the light of information covered in previous chapters. The genetic disorders that we will discuss here have somewhat arbitrarily been divided into diseases of lymphocyte development and immune dyscrasias involving aberrant lymphocyte activation. As will be obvious to most students, there is likely to be considerable overlap between the processes of development and activation. Mutations in *Rag-1* or *Rag-2*, for instance, that drastically compromise antigen receptor rearrangement may lead to a Severe Combined Immunodeficiency phenotype, while other mutations in these very same genes permit partial V(D)J recombinase activity and result in the very distinct clinical presentation of Ommen's syndrome.

Disorders of Lymphocyte Development

The last decade has seen the mechanistic unraveling of many members of a group of specific immunodeficiency disorders which were often lumped together before the causative genes for some of them were identified. Clinically, children with immunodeficiency disorders may present with with recurrent infections. Recurrent pyogenic infections with bacteria such as *Streptococcus pneumoniae* and *Hemophilus influenzae* are commonly seen in disorders of B cell development or function. In disorders in which T cell development or function is compromised, opportunistic pathogens such as *Pneumocystis carinii* may be responsible for symptoms and the likelihood of succumbing to intracellular pathogens such as *Mycobacteria* or viruses is greatly increased.

A number of inherited immunodeficiency disorders that affect more than one lineage are commonly divided into two sub-sets, the "Severe Combined" and "Combined" Immunodeficiencies (Table 1). While for simplicity's sake we maintain this classification, the distinction between "Severe Combined" and "Combined" immunodeficiencies is made on the basis of clinical rather than mechanistic considerations.

Severe Combined Immunodeficiencies

The Severe Combined Immunodeficiencies (SCID) in general represent diseases which are fatal early in life. They also represent a set of disorders which are ideal candidates for gene therapy and which may therefore prove completely curable in the next few decades. As a group, these deficiencies are noted in about 1 in 75,000 live births, are likely to prove lethal during the first year of life, and all forms involve a profound block in T cell differentiation. Infants present with opportunistic infections, often caused by live vaccines, and a failure to thrive. There at least eight variant forms of SCID.

Reticular Dysgenesis

As seen in Figure 1, some of these disorders represent defects in the development of early lymphoid progenitors prior to commitment to the T and B lineages. In patients with a form of SCID known as reticular dysgenesis, no T, B, NK, or myeloid cells are seen. The defect resembles that observed in the PU.1 knockout mouse in which, as in reticular dysgenesis, erythroid and platelet differentiation remain unaffected.

Table 1
Primary Immunodeficiencies involving defects in lymphocyte development

1. Defects in Lymphocyte development
    1.1. Severe combined immunodeficiencies
        a. Absence of all myeloid and lymphoid cells (Reticular dysgenesis)
        b. Absence of all lymphoid populations (Adenosine deaminase
            deficiency)
        c. Absence of T and NK cells (X-linked SCID; γc deficiency)
        d. Absence of T and NK cells (autosomal SCID; JAK-3 deficiency)
        e. Absence of T and NK cells (autosomal SCID:unknown
            mechanism)

    e. Absence of T and B cells (Alymphocytosis; *Rag* gene
defects)
    f. Absence of T and B cells (Alymphocytosis; non-RAG
recombination
     defect)
    g. Absence of T cells ($T^-B^+NK^+$ SCID; IL-7R deficiency)

   1.2 Combined Immunodeficiencies
    a. DiGeorge syndrome
    b. Purine nucleotide phosphorylase deficiency
    c. ZAP-70 mutations
    d. DNA ligase I deficiency
    e. CD3 ε deficiency
    f. CD3 γ deficiency
    g. HLA class II deficiency (CIITA, RFX5)
    h. Class I deficiency (TAP2)
    i. Ataxia telangiectasia
    j. Cartilage hair hypoplasia

   1.3. Humoral Immunodeficiencies
    a. X-linked agammaglobulinemia (Bruton's disease)
    b. Human surrogate light chain and μ heavy chain
mutants
    c. IgG subclass deficiencies
    d. κ chain deficiency
    e. X-linked hyper-IgM syndrome
    f. Hyper-IgM syndrome (autosomal)
    g. Selective IgA deficiency
    h. Common variable immunodeficiency

2. Disorders involving aberrant lymphocyte activation
    a. Wiskott-Aldrich syndrome
    b. Autoimmune lymphoproliferative syndrome
    c. "Accelerated phase" disorders
     c1. Chediak-Higashi syndrome
     c2. Familial hemophagocytic lymphohistiocytosis
     c3. X-linked lymphoproliferation
     c4. Immunodeficiency with partial albinism
    d. Omenn's syndrome
    e. Hyper-IgE syndrome
    f. Idiopathic disseminated mycobaterial infections

HSC → Megakaryocytes

E,G,M,L → Erythrocytes

RETICULAR DYSGENESIS ⊣

G, M, L → Granulocytes

M, L → Monocytes
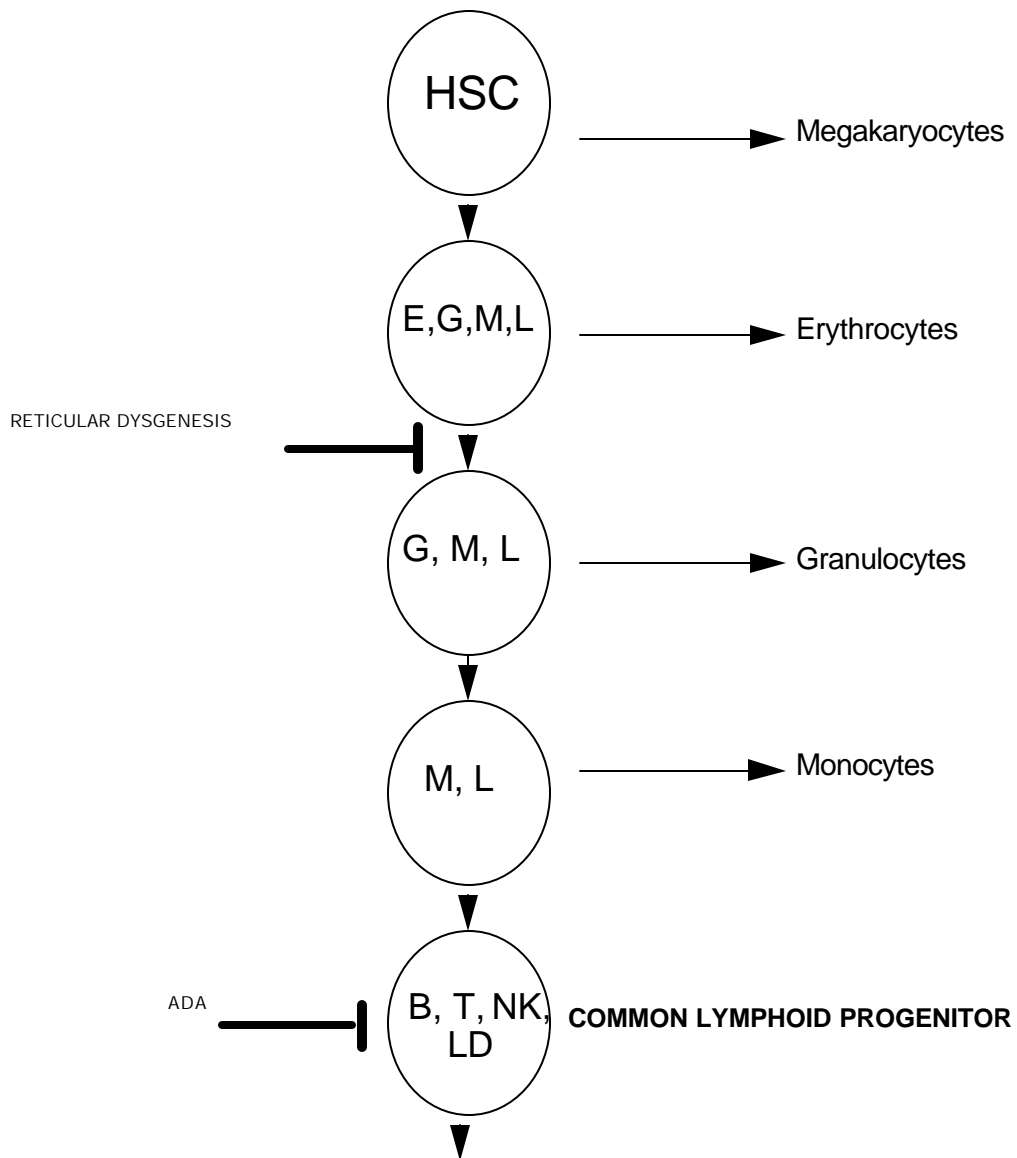
ADA ⊣ B, T, NK, LD **COMMON LYMPHOID PROGENITOR**

Figure 1. Some forms of SCID involve defects in progenitor cells.
The presence of platelets (P) and erythrocytes (E) in reticular
dysgenesis is reminiscent of the mouse PU.1 knockout. G refers to
granulocytes, M to macrophages, and L to lymphocytes. In ADA
deficiency the susceptible cell may be the common lymphoid
progenitor, although the most affected cell is probably a T cell
progenitor. LD refers to lymphoid dendritic cells.

Adenosine Deaminase Deficiency

A fairly common form of SCID occurs in infants with an
inherited deficiency in adenosine deaminase or ADA. During the
catabolism of purines (see Figure 2), phosphate residues are
removed from nucleotide monophosphates by nucleotidase. Inosine,
xanthosine and guanosine are further degraded by the action of

purine nucleoside phosphorylase or PNP and subsequently converted to uric acid. Intermediates in the degradation process may be reused to form nucleotides by salvage reactions. Adenosine and deoxyadenosine are not degraded in mammalian cells by PNP, but instead are deaminated by ADA to yield inosine and deoxyinosine. The latter are degraded by PNP and xanthine oxidase, the final product being uric acid. ADA has been crystallised and forms an eight stranded α/β barrel with the active site at the C-terminal end of the β barrel. There are eight different mutant forms of ADA that have been described in SCID patients all of which compromise the active site of the enzyme.

In the absence of functional ADA, deoxyadenosine is phosphorylated to produce very high levels of dATP. These high levels of dATP inhibit ribonucleotide reductase which is essential for dNTPsynthesis. DNA synthesis is thereby choked off and cells cannot proliferate. Patients with ADA deficiency have defects in the the generation of T, B, and NK cells suggesting a defect at the level of the putative common lymphoid progenitor, or in the early developmental stages of each of these three lineages. Indeed NK cell function is often unaffected in patients with ADA, and B cell development may be minimally compromised, suggesting that the defect may be largely confined to T cell precursors. Why are only these hematopoietic cells affected? It appears that the phosphorylation of deoxyadenosine occurs particularly efficiently in lymphoid progenitors and it is likely that the earliest cell in which toxic levels of dATP accumulate might be the common lymphoid progenitor cell, but that the most susceptible cel may be a T cell progenitor. The manifestations of ADA deficiency may not be restricted to lymphoid cells - patients also exhibit rib cage and other skeletal abnormalities, presumably in response to toxic effects of dATP and the inhibition of proliferation of other selected cell types.
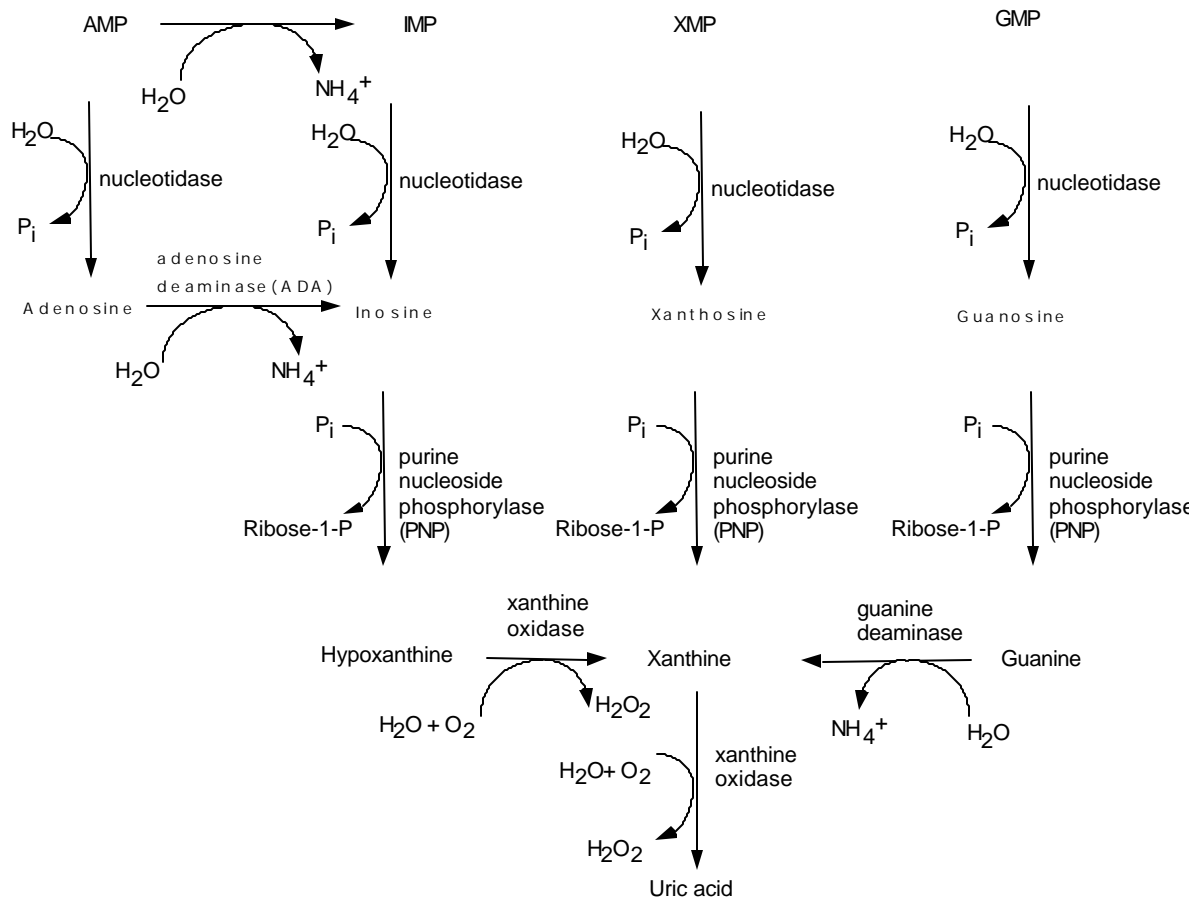
AMP → IMP   XMP   GMP

H_2O → NH_4^+

H_2O / P_i nucleotidase

H_2O / P_i nucleotidase

H_2O / P_i nucleotidase

H_2O / P_i nucleotidase

adenosine deaminase (ADA)

Adenosine → Inosine   Xanthosine   Guanosine

H_2O → NH_4^+

P_i → Ribose-1-P   purine nucleoside phosphorylase (PNP)

P_i → Ribose-1-P   purine nucleoside phosphorylase (PNP)

P_i → Ribose-1-P   purine nucleoside phosphorylase (PNP)

xanthine oxidase   guanine deaminase

Hypoxanthine → Xanthine ← Guanine

$H_2O + O_2$ → H_2O_2

NH_4^+ ← H_2O

$H_2O + O_2$ → H_2O_2 xanthine oxidase

Uric acid

Figure 2. An overview of purine catabolism in mammalian cells.Mutations
in adenosine deaminase result in the accumulation of phosphorylated dATP
in lymphoid progenitors which inactivate ribonucleotide reductase and
thus arrest cell cycle progression.While ADA deficiency affects B, T, and
NK cell development, mutations in purine nucleotide phosphorylase
primarily compromise T cell function.

   When ADA is injected intravenously it is cleared very
rapidly by the liver. However when PEG (polyethylene glycol) is
covalently coupled to ADA, the enzyme survives in the bloodstream
for one or two weeks and these adducts can be used to treat ADA
deficiency fairly effectively. Preliminary attempts at gene
therapy have yielded promising results, and it is very likely
that this will prove to be the therapeutic intervention of choice
in the near future.

X-linked SCID

   The common γ chain (γc) of the IL-2, IL-4, IL-7, IL-9, and
IL-15 receptors is encoded by an X-linked gene (located at Xq12-
13.1). Defects in this gene lead to an X-linked form of SCID in
which T and NK cell development are compromised, but there is no
defect in B cell generation. This is the most common form of

SCID, and a large variety of mutations have been discovered in γc. This is a lethal disease, and there is clearly a very high rate of new mutations being generated. In man IL-7 is required for T cell development but is not critical for B cell development. IL-15 is required for NK cell development and the combined loss of IL-7 and IL-15 derived signals leads to the failure of development of these lineages (Figure 3).

Autosomal recessive SCID with JAK-3 deficiency
Mutations in an autosomal gene encoding the JAK3 kinase result in a very similar phenotype to that seen in X-linked SCID. This is not at all surprising since JAK-3 and γc are functionally linked (Figure 3) and identical phenotypes are seen in mice in which these genes are separately targeted. JAK-1 is also a critical player in this pathway in the mouse, but mutations in JAK-1 have yet to be described in patients with autosomal recessive forms of SCID. IL-7 plays a demonstrable role in B cell development in the mouse but not in man, and this accounts for the differences in phenotype seen between the human disorders involving γc and Jak-3 and the corresponding knockout mice lines.

Autosomal recessive SCID with IL-7R deficiency
In a form of SCID in which there is a defect in T cell development with normal numbers of B and NK cells (T-B+NK+ SCID), inherited mutations in the gene encoding the IL-7Rα chain have been described. This disease underscores the fact that in humans IL-7Ra derived signals are not required for B cell development, and that IL-7 is not required for NK cell development in mouse or man (although IL-15 is crucial).
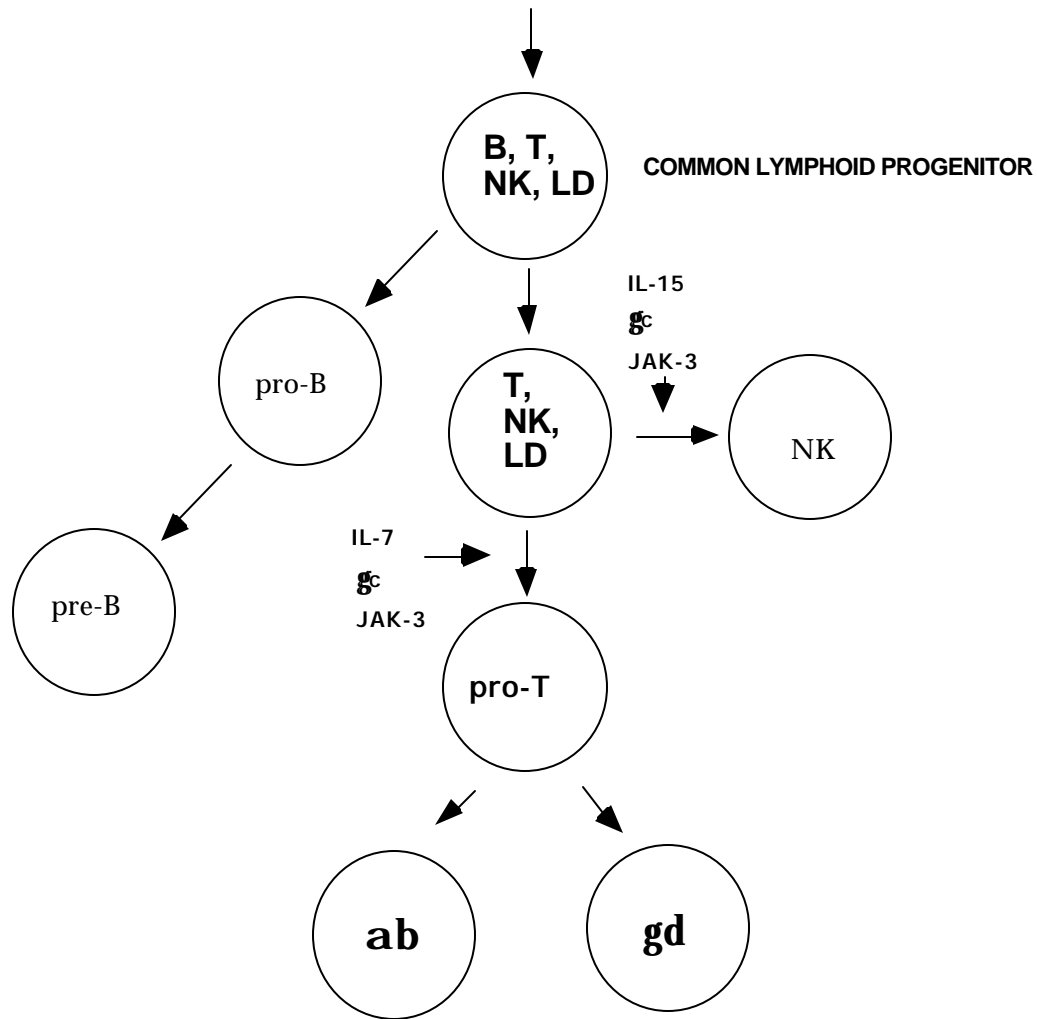
Figure 3. IL-7 and IL-15 influence the development of T cells
and NK cells respectively in humans. Signals downstream of the
receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 are transduced
via γc, Jak-3 and probably JAK-1 as well. LD refers to lymphoid
dendritic cells.

Other autosomal recessive forms of SCID

     The four autosomal recessive forms of SCID considered above
are reticular dysgenesis, Jak- 3 deficiency, ADA deficiency, and
an IL-7Rα chain defect. Two autosomal recessive forms of SCID
have been described in which B and T cells are not seen. In one
of these forms mutations in *Rag-1* or *Rag-2* severely compromise
V(D)J recombination. In an additional form, V(D)J recombination
is compromised but no mutations have as yet been described either
in the Rag genes or DNA repair related components such as DNA-PK,
Ku-70, or Ku-80. DNA PK is defective in SCID mice and in an
equine form of SCID seen in Arabian foals. Certain mutations in
the *Rag-1* and *Rag-2* genes result in partially compromised V(D)J

recombination and an unusual clinical phenotype described as Omenn's syndrome, which will be discussed later in this chapter.

A form of autosomal recessive SCID similar in phenotype to X-linked SCID and Jak-3 deficiency (with no T or NK cells) has been described. The defective gene in this form has not yet been identified, but it is likely to encode a protein in the γc/JAK-1/JAK-3 pathway (other than JAK-3 or γc).

"Combined" Immunodeficiencies

This category of disorder is characterized by a defect usually in T cell development which does not lead to as severe a phenotype as is seen in SCID. Defects either in B cell development or function also may be seen (often because of the absence of T cell help). As discussed earlier, the distinction between SCID and "CID" is operational, and there is considerable overlap between these groups of diseases.

DiGeorge's syndrome

DiGeorge's syndrome is characterized by thymic and parathyroid hypoplasia. In its most severe form it can resemble SCID but the defect does not involve the B lineage. This syndrome results from dysmorphogenesis of the third and fourth pharyngeal pouches. The defect in thymic development results from a presumed failure of the neural crest mesenchyme to induce morphogenetic changes in the third pharyngeal pouch derived endoderm. The gene that is defective in the DiGeorge syndrome has been located to 22q11 but remains to be cloned.

Purine nucleoside phosphorylase deficiency

Nezelof's syndrome is a form of combined immunodeficiency in which B cell development is normal but T cell development is impaired. A large number of patients with this syndrome have a defect in an enzyme that is critical for purine degradation, purine nucleoside phosphorylase or PNP (see Figure 12.2). Why exactly PNP deficiency targets T cell development is unclear. Inhibition of PNP by pharmacological means can prevent the hyperactivation of T cells seen in some autoimmune diseases but the mechansism by which PNP activity is linked to T cell development or T cell activation remains to be elucidated.

Mutations Zap-70 and CD3 genes

A form of T cell deficiency seen in man results from mutations in the Zap-70 tyrosine kinase, a member of the Syk family. These mutations occur in the kinase domain of this enzyme (Figure 4)

ZAP-70

SH2-N	SH2-C	Kinase

10 ... 102   163 ... 255   338 ... 592
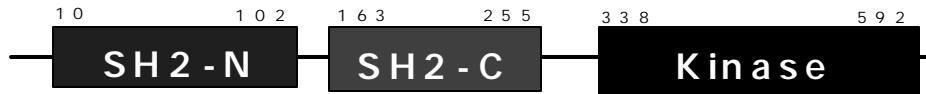
Mutations in human SCID/CID

1. A 13 bp deletion from
   nucleotides 1719-1731
2. Insertion of LEQ at position 542
   (nucleotide 1832)
3. R to S at position 518

ZAP-70 deficiency

● Recurrent and opportunistic infections in first year of life

● CD8 cells virtually absent

● CD4 cells non-reactive

(CD4 binds better to Lck than CD8; ?some Syk activation in CD4 cells)

Figure 4. Mutations in ZAP-70 compromise human CD8 T cell development. In man the primary defect is in CD8^{+} T cell generation

Unlike Zap-70 null mice which lack both CD4 and CD8 T cells because of a failure of TCR signaling during positive selection II, in man Zap-70 mutations results only in a failure of CD8 T cell development. However while CD4 cells do emerge in patients with inherited defects in ZAP-70, these cells cannot be readily activated in response to TCR triggering. These children have recurrent opportunistic infections in the first year of life. They may sometimes be categorized as SCID patients and these distinctions are somewhat arbitrary. Why do patients with ZAP-70 defects not have a problem in positively selecting CD4 T cells? It has been suggested that CD4 more readily associates with Lck than CD8, and that as a result, ZAP-70 is more readily activated during positive selection II in CD4 cells than in CD8 cells. Alternatively Syk may be more readily activated in CD4 T cells than in CD8 cells and may thus compensate for the absence of Zap-70 in the former.

Defects in CD3ε and CD3γ also result in defects in T cell development presumably because they compromise signaling via the pre-TCR and TCR.
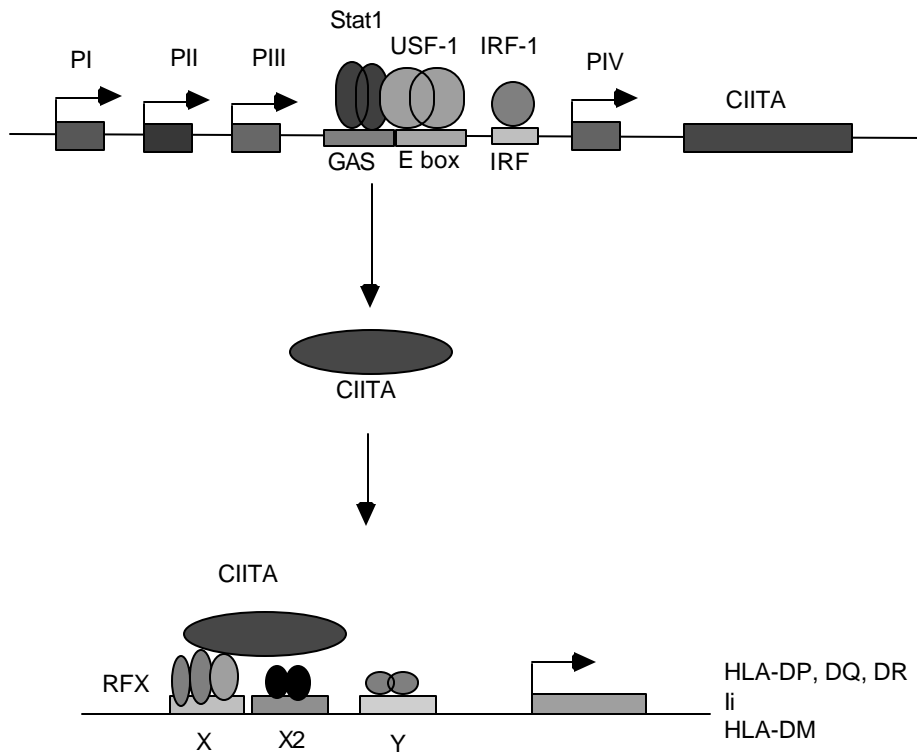

T cell immunodeficiencies associated with DNA Ligase I mutations
It is likely, as discussed above, that some SCID or CID phenotypes will be associated with mutations in genes that are involved in Double Strand Break repair such as Ku70 and Ku80. Defects in T cell development have been noted in isolated patients who have inherited mutations in DNA ligase I, which may be required during V(D)J recombination (although DNA ligase IV appears to be the major DNA ligase involved in this process).

The "Bare lymphocyte syndrome": MHC class II deficiency
As expected inherited disorders in which MHC class II expression is compromised result in a defect in CD4 T cell development, and defects in MHC class I expression result in abnormalities in CD8 T cell positive selection. MHC class II expression defects have been noted in patients with the bare lymphocyte syndrome who have inherited defects in transcriptional regulators of MHC class II (HLA DP, DQ, and DR) expression. These patients also fail to express the invariant chain (Ii) and HLA-DM, a class II like heterodimer which facilitates the exchange of the invariant chain derived CLIP peptide that remains bound to MHC class II proteins, with peptides derived from the proteolysis of endocytosed proteins. The failure to express HLA class II molecules in the thymic cortex during positive selection results in an absence of CD4 T cells. Patients may belong to any one of four complementation groups. Three of the four regulatory genes involved in this syndrome, CIITA, RFX5, and RFXAP, have been isolated. As seen in Figure 5, RFX5 and RFXAP are components of the multimeric RFX complex which binds to the X box in MHC class II, Ii, and HLA-DM promoters. Although RFX is ubiquitously expressed its activity depends on a co-activator CIITA which may be expressed constitutively in professional APCs but can also be induced by IFN-g in certain cell types. CIITA is regualted by multiple promoters which result in distinct CIITA transcripts with different first exons. The promoter PI is responsible for constitutive expression in dendritic cells and the promoter PIII is required for constitutive B cell expression. The PIV promoter regulates IFN-γ mediated induction of CIITA. Regulation of this promoter depends on the binding of phosphorylated STAT1 to a GAS site, which cooperates with the ubiquitously expressed bHLH protein USF1 which binds to an adajcent E-box. STAT1 is phosphorylated by JAK1/2 as a consequence of IFN-γ signaling. One

of the other regulatory elements is an IRF-1 binding site. During B cell development the extinction of MHC class II expression observed in plasma cells is also achieved by repression of CIITA. An inherited deficiency in MHC class-I (HLA-A,-B, and -C) gene expression is noted in patients who have inherited defects in the expression of the TAP-2 peptide transporter, an ABC cassette containing protein which, as part of the TAP1/TAP2 heterodimer,

Stat1

PI   PII   PIII   USF-1   IRF-1   PIV   CIITA

GAS   E box   IRF

CIITA

CIITA

RFX   X   X2   Y   HLA-DP, DQ, DR
Ii
HLA-DM

is responsible for the translocation of proteasomally cleaved peptides from the cytosol into the endoplasmic reticulum.
Figure 5. Regulation of HLA Class II gene expression.

Immunodeficiency associated with Ataxia Telangiectasia
       One fairly complex and poorly understood form of combined immunodeficiency is observed in patients with ataxia telangiectasia. Patients present with a host of neurologic, immunologic, endocrine, cutaneous and hepatic abnormalities. These include progressive cerebellar ataxia, oculocutaneous telangiectasias, chronic sinopulmonary disease, a high incidence of malignancy, and immunodeficiency. Between 50-80% of patients may present with a selective absence of IgA. IgE and IgG levels are frequently decreased. Cell mediated immunity is impaired and the thymus has been noted to be hypoplastic. The gene that is defective in ataxia telangiectasia is located on 11q22-23 and encodes a protein known as ATM for Ataxia Telangiectasia Mutated. This protein is a member of the PI-3 kinase family and is a dual

serine/threonine protein kinase/lipid kinase. ATM is induced by
DNA damage and may contribute to the induction of p53 and the
induction of apoptosis (see Figure 6) thus functioning as part of
a cell cycle checkpoint. ATM can bind to p53 and phosphorylate it
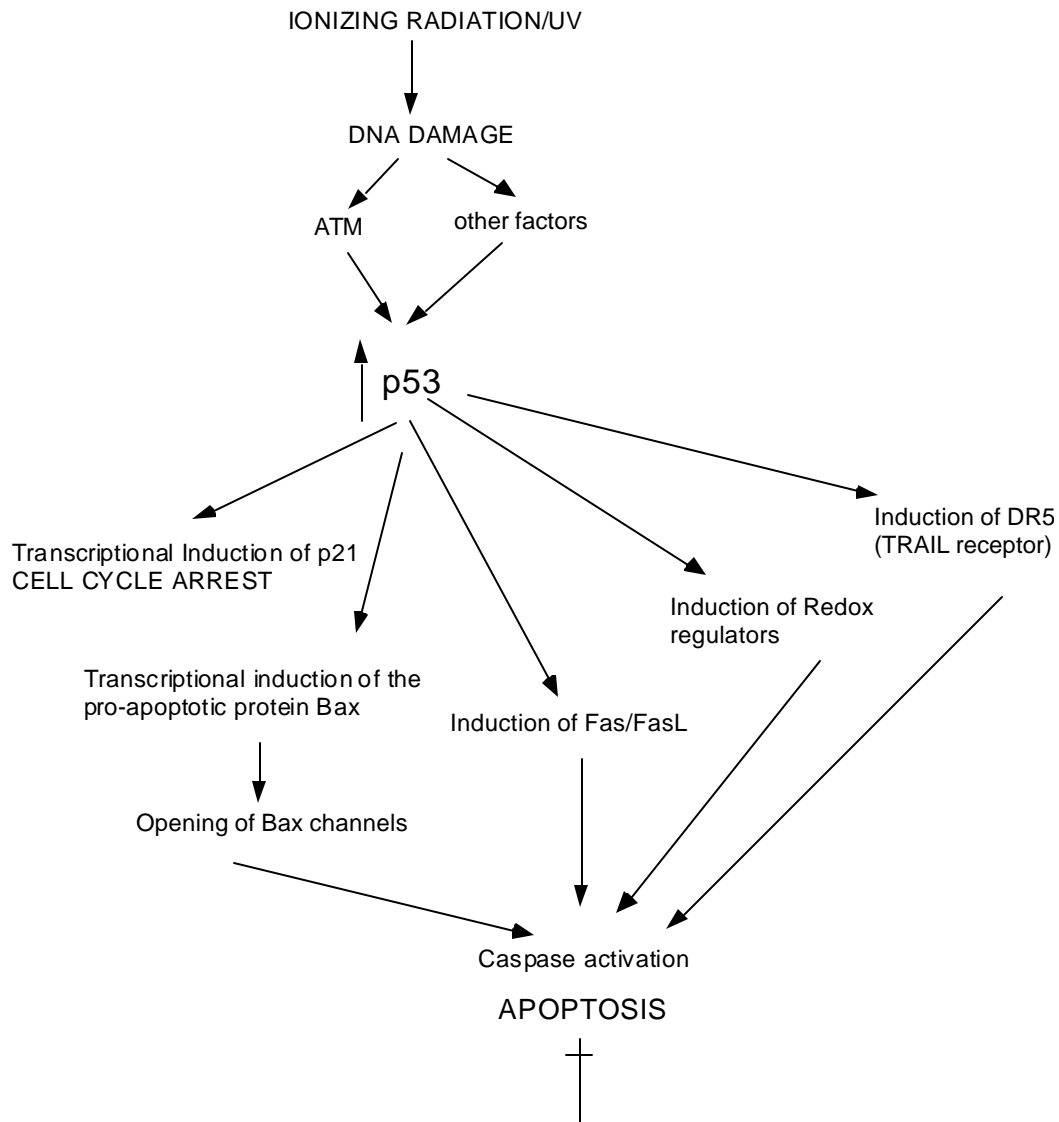on serine-15, thus activating and stabilizing p53.

IONIZING RADIATION/UV

DNA DAMAGE

ATM   other factors

p53

Transcriptional Induction of p21
CELL CYCLE ARREST

Induction of DR5
(TRAIL receptor)

Induction of Redox
regulators

Transcriptional induction of the
pro-apoptotic protein Bax

Induction of Fas/FasL

Opening of Bax channels

Caspase activation

APOPTOSIS

Figure 6. ATM may be part of a DNA damage checkpoint involving
p53. ATM binds to p53 ans phosphorylates it on serine-15, thus
stabilizing the p53 protein.

In a poorly understood way this kinase is believed to be
critical for maintaining the integrity of recombination. While
V(D)J recombination itself is not impaired, the integrity of the

joining process may be compromised leading to an extremely high frequency of translocations in B and T lymphocytes. While this presents a rather unsatisfying explantion at present for the immunodeficiency and cancer seen in this disease, this disease and the ATM gene are being very aggressively studied at present and answers are better likely to be at hand in the near future.

There is a potential link between V(D)J recombination and the maintenance of chromosomal integrity and this is reflected in a few genetic disorders that result in immunodeficiency as well as in chromosomal translocations and the malignant transformation of lymphocytes.

The *nude* mouse presents with a naturally occurring defect in thymus development and has been discussed in detail in Chapter Nine.

Humoral immunodeficiencies

In disorders in which B cell development is primarily impaired (see Table 1), antibodies are not made and patients may be particularly susceptible to bacterial infections.

X-linked agammaglobulinemia

The earliest developmental stage at which B cell ontogeny is blocked in a human disease is at the pro-B to pre-B transition, presumably because of compromised pre-B receptor signaling. In **X-linked agammaglobulinemia (XLA)** or **Bruton's disease** numerous different mutations have been described in Btk a protein tyrosine kinase discussed in Chapters Four and Seven. These mutations include point substitutions and deletions and can result in a range of altered proteins or no protein at all. In different XLA patients, point substitutions have been described in the PH domain, the SH2 domain, the SH3 domain, and the kinase domain, suggesting important functional roles for each of these domains. The severity of the disease presumably depends on the extent to which catalytic activity is compromised and in some patients may be influenced by the epistatic role played by unidentified "modifier" genes. In its most severe form no peripheral B cells are seen and after the first year of life, boys carrying this defect have recurrent pyogenic infections usually with *H.influenzae* and *S.pneumoniae*. Very low levels of serum immunoglobulins are observed and the treatment at present primarily involves frequent injections of polyclonal human serum immmunoglobulins. Gene therapy is also being considered. The primarily defect in this disease is almost certainly linked to the failure to generate pre-B receptor derived checkpoint signals during development. It is particularly interesting that a phenotype similar to that observed in XLA has been described in children carrying mutations in the human 14.1 gene which encodes

the human homolog of the λ5 gene, an important component of the pre-B receptor. A similar autosomal recessive agammglobulinemia is also observed in females as well as males with mutations that compromise the structure and function of the µ Ig heavy chain gene.

It is worth noting that in the mouse Btk is not absolutely required for Positive selection I at the pre-B receptor mediated checkpoint. However Btk does participate in peripheral B cell maturation as described in Chapter Seven. A naturally occurring Btk mutation occurrs in the Xid mouse which has been discussed in some detail in Chapter Seven. In some patients with XLA, milder phenotypes which mimic those seen in the Xid mouse might reflect defects in peripheral B cell maturation and survival.

The X-linked hyper-IgM syndrome

Another X-linked disease in which B cell function is compromised is the X-linked hyper-IgM syndrome. In this disease young boys present with recurrent pyogenic infections generally with *H.influenzae* and *S.pneumoniae*, just as in patients with XLA, but may also have opportunistic infections particularly involving *Pneumocystis carinii*. These latter opportunistic infections suggest a defect in cell mediated immunity. In this syndrome mutations have been described in the X-linked gene that encodes the CD40 ligand. CD40L on activated T cells is critical for T-dependent activation of B cells and for the initiation of B cell proliferation, class switching, germinal center formation and somatic hypermutation as discussed in Chapter Eight. As a result boys with this syndrome tend to make largely IgM antibodies and do not expand their repertoires via affinity maturation. CD40L also participates in T cell activation (Figure 7) as discussed in Chapter Ten, resulting in the cell mediated immune defects noted in patients with this syndrome. The interaction of CD40L with CD40 not only results in the delivery of signals to the B cell but also may lead to signal generation in the T cell. In addition the activation of dendritic cells and other APCs via CD40 signaling can induce costimulatory ligands such as B-7 proteins. These activated cells may facilitate the release of IL-12 by macrophages and the activation of a TH1 type of response. In patients with the X-linked hyper-IgM syndrome, the activation of T cells, probably as a consequence of defective APC activation, is compromised.
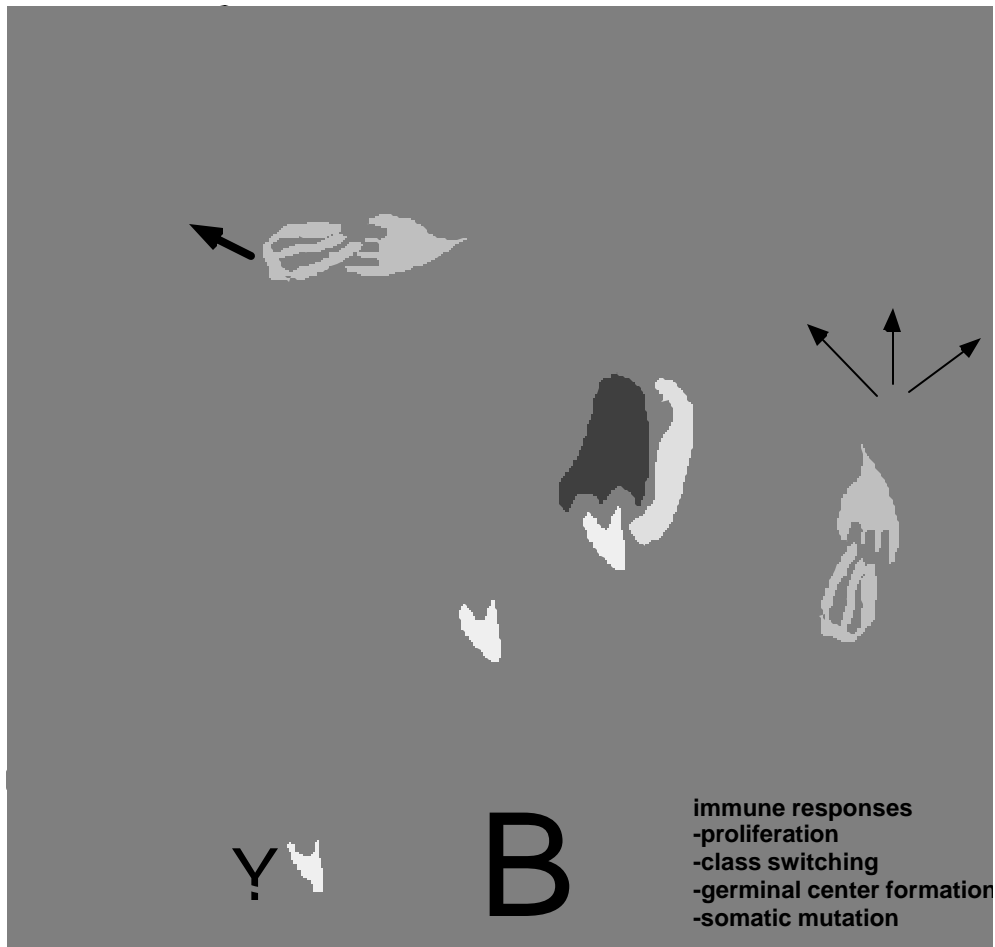
Figure 7. CD40 L is required both for the activation of B cells and T cells. Triggering of CD40 on dendritic cells permits the induction of B7 molecules which facilitate the generation od Signal Two for proper T cell activation (See discussion on "licensing" in Chapter Ten).

Disorders involving aberrant lymphocyte activation

In a number of inherited immune dyscrasias, the major defect appears to be inappropriate activation of the immune system. In a sense all allergic and autoimmune disorders might be lumped in this category but they generally involve more subtle abnormalities of the immune system and represent multi-gene disorders which are not inherited in a Mendelian fashion. In the diseases that we will consider here, mutations in genes involved in some aspect of lymphocyte development result in the generation of large numbers of activated lymphocytes which contribute to pathology.

The Wiskott-Aldrich syndrome

The Wiskott-Aldrich syndrome is an X linked disease characterized by thrombocytopenia, defective platelet activation,

pyogenic and opportunistic infections, and eczema. IgM levels tend to be low, IgA and IgE levels are generally elevated and IgG levels are usually in the normal range. There is a failure to respond to polysaccharide antigens and T cells have signaling and cytoskeletal defects. The X linked gene that is defective is this disease encodes a protein which is known as WASP (Wiskott Aldrich Syndrome Protein). WASP is a proline rich protein with multiple sites capable of interacting with SH3 domains. The WASP protein interacts with the Rho family small GTPase, Cdc42, with Src family kinases, with the Nck adaptor protein, with proline serine threonine phosphatase interacting protein (PSTPIP), and with a protein known as WIP (WASP interacting protein) among others. Cdc42 is involved in actin rearrangements as is WIP and PSTPIP. WASP is a homolog of the *S.cerevesiae* cortical actin rearrangement inducing protein Bee1 and in activated lymphocytes, platelets, and other cells, the function of WASP appears to be to help orchestrate the rearrangement of cortical actin. A critical function of Cdc42 and of WASP in T cells is the formation of a polarized synapse like interface with antigen presenting cells (see Chapter Four). Almost all of the defects seen in the Wiskott Aldrich syndrome can potentially be tied to the failure of WASP to help reorganize cortical actin.

It is possible that in B cells triggered by T-independent polysaccharide antigens, Btk signaling involves the tyrosine phosphorylation of WASP. A site on WASP that appears to be tyrosine phosphorylated *in vivo* closely resembles the autophosphorylation site on Btk itself. WASP patients do have defective responses to polysaccharide antigens and this B cell defect closely resembles that seen in the Xid mouse.

Autoimmune Lymphoproliferative Syndrome

Another interesting but very rare disorder involving aberrant lymphocyte activation is the Autoimmune Lymphoproliferative Syndrome in which Fas mutations have been found. Infants and young children with this disease present with lymphadenopathy, splenomegaly, and hypergammaglobulinemia. These lymphoid organs are overpopulated with $CD3^+CD4^-CD8^-$ T cells. In addition, common features of this syndrome include episodic autoimmune anemias, thrombocytopenia, autoantibody mediated neutropenias, and urticarial rashes. This syndrome closely mimics the phenotype of naturally occurring mutations in the mouse of of Fas (in the *lpr* mouse) and the Fas ligand (in the *gld* mouse) briefly discussed in our discussions on apoptosis. Not only do activated T cells not get eliminated by Activation Induced Cell Death, but as explained in an earlier lecture, bystander and potentially self reactive B cells which should normally be

eliminated via Fas signals proliferate and may contribute to autoimmune manifestations.

There are two interesting features to be noted about the inheritance of this extremely rare disease. The mutations in Fas are generally observed in hetrozygotes, and homozygous mutations are rare. Given that Fas is a trimeric protein , the likelihood that all three subunits in a given trimer will be "normal" in a heterozygote (assuming equivalent abundance of both normal and mutant proteins) is only 1/8. The second interesting feature related to the inheritance of this rare disease, is that the heterozygous mutant chromosome is usually transmitted from a normal parent. This indicates that modifier genes play an important role in contributing to this syndrome. Interestingly the genetic background is also a critical contributor to the autoimmune phenotype of *lpr* and *gld* mice.

Disorders involving a post-infectious "accelerated phase"

A small number of genetic disorders result in what appears to be an accelerated and uncontrolled end-state of TH1 cell and macrophage activation probably following a viral infection. In these diseases, which include the Chediak-Higashi syndrome, familial hemophagocytic lymphohistiocytosis, partial albinism with immunodeficiency, and possibly the X-linked lymphoproliferative syndrome, a defect in NK cell activity is also frequently seen. The common feature in this otherwise diverse sub-set of diseases is probably a basic inability to counter viral infections, and the consequent triggering of an accelerated phase of TH1 type T cell activation.

The *Chediak-Higashi syndrome* (CHS) in man has a murine counterpart, the *beige* mouse. This syndrome is characterized by partial cutaneous and ocular albinism, defective CTL and NK cell activity, increased susceptibility to infections, and the accumulation in many cells of large intracellular granules. There is a defect in granule discharge (accounting for the defects in CTLs and NK cells) and in the fusion of endosomes with primary lysosomes. The defective gene on human chromosome 1 has been identified and is believed to encode a cytosolic protein that is involved in a vesicular trafficking function - probably regulating the guided movement of vesicles along microtubules during endocytosis and exocytosis. Most patients with CHS develop an accelerated phase which generally leads to death. During the accelerated phase T cells and macrophages are activated and cytokine production spins out of control.

A similar accelerated phase response is observed in an autosomal recessive condition known as *familial hemophagocytic lymphohistiocytosis* (FHL). FHL is characterized by polyclonal T lymphocyte and macrophage activation that begins in early

childhood and invariably has fatal consequences. Macrophage activation presumably occurs because T cells secrete large amounts of interferon-γ. The serum levels of IFN-γ, TNF, and IL-1 are greatly elevated. These activated macrophages ingest red cells and along with the activated T cells infiltrate the liver, spleen, bone marrow, and the central nervous system. It has been suggested that this syndrome in some ways resembles the phenotype of CTLA-4 $^{-/-}$ mice, but CTLA-4 mutations have yet to be described in this or any other clinical condition.

X-linked lymphoproliferative syndrome
     In the *X-linked lymphoproliferation* (XLP) syndrome, also known as Duncan's syndrome, young boys are prone to succumb to Epstein-Barr virus infections after entry into an accelerated phase. The causative gene on Xq25 encodes a small protein called SAP (for SLAM associated protein). SAP contains an SH2 domain but not much else. SLAM, is a costimulatory molecule found on activated T cells (CD45RO cells), thymocytes, and some B cells. SLAM is activated by homotypic interactions, can induce lymphocyte proliferation, and can facilitate the generation of Th1 responses. This member of the Ig superfamily is structurally related to CD2 and its cytoplasmic tail contains four potential tyrosine phosphorylation sites. Some of these sites, even in an unphopshorylated state can bind to SAP with a very high affinity. SAP presumably prevents other SH2 containing signaling molecules from being recruited to one or more of these sites. The cytoplasmic tail of SLAM can potentially recruit a range of SH2 domain containing proteins, and why exactly the absence of SAP leads to immunodeficiency or uncontrolled B cell proliferation following EBV infection is unclear. The primary defect in the disease could involve T cells, or B cells, or both. Although SAP is expressed primarily in B cells, it is also expressed at high levels in germinal center B cells. Exactly how SAP regulates immune function and lymphocyte proliferation is curently being investigated. It is possible that the underlying defect is in a mechanism involved in viral clearance by T cells, or may involve the dysregulation of proliferation in B cells. Most of the immunological abnormalities noted in this disease may be manifest after EBV infection. This disorder may therefore fit into the broader "accelerated phase" category being discussed above.

Omenn's syndrome
     In this rare autosomal recessive disease infants present with severe erythrodermia, alopecia, diarrhea, and a failure to thrive. Large numbers of activated T cells infiltrate the skin, the gut epithelium, and the lamina propria. While there is a profound depletion of T and B lymphocytes in the blood and lymph

nodes, the massive tissue infiltrations of T cells in this disease appear to be generated from a few T cell clones have taken over the whole immune system. These few T cells may be $\gamma\delta$ T cells, or $\alpha\beta$ T cells which are CD4^{+}, or CD8^{+} or DN. Functionally some of the oligoclonal T cell expansions may be of the TH2 type since eosinophilia and high blood levels of IL-4 and IL-5 are characteristically noted.

The underlying defect in a number of families with Omenn's syndrome is an inherited missense mutation in the *Rag-1* or *Rag-2* gene that contributes to an incomplete abrogation of V(D)J recombination. This partial defect results in an almost complete block in B and T cell generation with the occassional developmental escape of a few T cell clones. Some of these T cells are apparently driven to unremitted expansion in these immunodeficient infants and this results in a "TH2" type accelerated phase disease with fatal consequences.

Disorders involving cytokines and cytokine receptors

It is likely that the coming years will see the identification of an increasing number of cytokine and cytokine receptor mutations in a range of disorders involving lymphocyte development or function. Defects have been discovered in the genes encoding the IFN-γRI and the IL-12Rβ2 chain in patients with "idiopathic" disseminated mycobacterial and *Salmonella* infections. Both these receptors are required for driving the Th1 versus Th2 decision . The IFN-γR is also required for macrophage activation in the course of Th1 type responses. Defects in the Il-7R and in IL-15R signaling have been discussed above.

Summary and Perspective

Although there are some differences in the details of lymphocyte development as studied in the mouse and in humans, the genes and mechanisms involved in lymphoid ontogeny are remarkably similar in these two species. It is clear that the enormous amount of information on lymphocyte development that has been obtained from knockout and knock-in strategies in the mouse will continue to grow. The identification of molecular defects that lead to disorders of human lymphocyte development has only just begun! But what a beginning it has been!

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