CURRENT TOPIC

Severe combined immunodeficiency—molecular pathogenesis and diagnosis

H B Gaspar, K C Gilmour, A M Jones

Severe combined immunodeficiencies (SCID) are a heterogeneous group of inherited disorders characterised by profound abnormalities in T, B, and natural killer cell development and function.¹ They arise from a variety of molecular defects, and the deficits in both cell mediated and humoral immunity lead to similar presentations in all the defined conditions. Children characteristically present with failure to thrive, recurrent infections, and increased susceptibility to opportunistic infection. The age of presentation is variable but occurs typically between 3 and 6 months when the protective effect of maternally transmitted immunoglobulin has diminished, although atypical and late presentations are well described.

Over the past 10 years there have been enormous advances in the understanding of the molecular basis of the different forms of SCID (see table 1). These have led to several improvements in diagnosis and management. Firstly, unambiguous assignment of a molecular diagnosis is now possible in many cases. This is particularly important in children who have evidence of combined (cellular and humoral) immunodeficiency, but with “milder” clinical phenotypes than infants with classical SCID. Some of these children are found to have identical molecular defects to those causing SCID, and in these cases the long term outlook is now known to be poor enough to justify bone marrow transplantation (BMT) at an early stage. Secondly, accurate carrier detection and first trimester prenatal diagnosis are possible in any family where the precise

mutation has been defined. In some cases pre-natal diagnosis of an affected fetus may not lead to termination of pregnancy, but can allow preparation for BMT early in the neonatal period, or even in utero in selected cases.² Thirdly, knowledge of the genetic defect has allowed a greater understanding of the molecular pathogenesis of the disease with the possibility of designing more rational therapies in some cases, and for developing strategies for somatic gene therapy.^{3,4}

Even before a molecular defect is identified, analysis of the immunological profile can provide an indication of the nature of the underlying genetic abnormality. Nearly all cases of SCID have very low or absent numbers of T lymphocytes. Patients are then grouped into those who have B lymphocytes (T-B+ SCID) and those who do not (T-B- SCID). Further subclassification can then be made according to the presence or absence of natural killer (NK) cells. For instance SCID caused by gamma chain (γ_c) or JAK3 deficiency has a characteristic T-B+ NK- profile (see fig 1). In all cases, the absolute lymphocyte count may be useful in making the diagnosis of SCID. Very low or absent counts (less than $2.7 \times 10^9/l$) may be seen in the T-B- forms and in T-B+ SCID, the lymphocyte count is usually below the lower end of the age related range.

SCID caused by abnormalities in the common gamma chain or JAK3

Of the different molecular defects that result in SCID, the most common is the X linked form

Molecular Immunology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK
H B Gaspar

Clinical Immunology Laboratory, Camelia Botnar Laboratories, Great Ormond Street Hospital for Children NHS Trust, London, UK
K C Gilmour

Department of Immunology, Great Ormond Street Hospital for Children NHS Trust
A M Jones

Correspondence to:
Dr Gaspar
h.gaspar@ich.ucl.ac.uk

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Table 1 Major types of SCID and their genetic defect

Disorder (year of definition of molecular basis)	Chromosomal location	Gene	Diagnostic tests other than direct mutation analysis
X linked severe combined immunodeficiency (1993)	Xq13	Common γ chain (γ_c)	γ_c expression by FACS analysis
Adenosine deaminase (ADA) deficiency (1983)	20q12–13	Adenosine deaminase	Red cell ADA levels and metabolites
Purine nucleoside phosphorylase (PNP) deficiency (1987)	14q11	Purine nucleoside phosphorylase	Red cell PNP levels and metabolites
Recombinase activating gene (RAG 1&2) deficiency (1996), Omenn's syndrome (1998)	11p13	RAG1 and RAG2	
T cell receptor deficiencies (1987)	11q23	CD3 ζ /CD3 ϵ	
Zap70 deficiency (1994)	2q12	ZAP-70	ZAP-70 expression
JAK3 deficiency (1995)	19p13	JAK3	JAK3 expression/signalling
IL-7 receptor deficiency (1998)	5p13	IL-7 receptor α	IL-7 receptor α expression
MHC class II deficiency (1993)	16p13	CIITA	HLA-DR expression
(1998)	19p12	RFX-B	
(1995)	1q21	RFX5	
(1997)	13q13	RFXAP

Figure 1 Immunophenotypes in SCID.

(X-SCID) which arises from defects in γ_c .⁵ This molecular defect results in the absence of T and NK cell development but normal B cell numbers, although recent evidence suggests there are intrinsic γ_c mediated defects of B cell function.⁶ A similar, though much rarer, clinical and immunological phenotype arises from an autosomal recessive defect in the gene encoding the tyrosine kinase JAK3 (Janus associated kinase 3).^{7,8} γ_c was initially identified as a component of the high affinity interleukin 2 receptor (IL-2R), but is now known to be an essential component of the IL-4, -7, -9, and -15 cytokine receptor complexes.⁹ Stimulation of the receptor complex by cytokine results in the heterodimerisation of the receptor subunits and phosphorylation of the JAK3 molecule which binds specifically to the γ chain subunit. Tyrosine phosphorylated JAK3 in turn phosphorylates one of the STAT (signal transducers and activators of transcription) family of transcription factors which then dimerises and translocates to the nucleus where it binds to specific sites to initiate transcriptional events¹⁰ (fig 2). The specific function of the different cytokines can

Figure 2 γ_c /JAK3 signalling pathway.

explain the immunological profile of XSCID and JAK3 SCID. Data from in vitro studies and from “knockout” mice models have shown that functional IL-7/IL-7R and IL-15/IL-15R mediated signalling pathways are essential for normal T and NK cell development respectively.^{11,12} Abnormalities in IL-2 and IL-4 signalling may further explain the functional B cell defects.

Prior to the identification of the genetic defects, diagnosis was based on family history and clinical and immunological profile. Linkage analysis and examination of X inactivation pattern in T cells of female relatives (a unilateral pattern is seen in female carriers) was used to guide diagnosis and carrier status but could only offer a degree of probability. These techniques have largely been replaced by direct analysis of the γ_c and JAK3 genes.^{13,14} If a mutation is identified, carrier assessment for female relatives can be made with absolute certainty and accurate prenatal diagnosis can be offered. More rapid tests based on the expression patterns and function of the mutant γ_c or JAK3 protein are also now available for diagnosis of affected infants. Approximately 65–90% of children with X-SCID have abnormal expression of γ_c on the surface of mononuclear cells¹⁵ (our own unpublished data support this), allowing confirmation of the molecular diagnosis by flow cytometric analysis of peripheral blood mononuclear cells (fig 3). In infants affected by T-B+NK SCID who have normal γ_c expression, further dissection of the signalling pathway can now be undertaken. IL-2 stimulation of mononuclear cells results in tyrosine phosphorylation of JAK3 at specific tyrosine based motifs. A monoclonal antibody directed against phosphotyrosine residues can be used to show JAK3 activation, so abnormalities in this signalling pathway can be detected at a protein level prior to genetic analysis. The variability in clinical presentation in these forms of SCID and especially in JAK3 deficiency again underlines the need to identify the molecular defect so that earlier referral for bone marrow transplantation can be made. With the development of successful somatic gene therapy protocols for X-SCID it is again essential that a rapid molecular diagnosis is made.³

SCID caused by abnormalities in purine metabolism

Approximately 20% of all cases of SCID arise from an autosomal recessive deficiency of adenosine deaminase (ADA), a ubiquitously expressed “housekeeping” enzyme that is required for the degradation of adenosine and deoxyadenosine (dAdo) following DNA breakdown. ADA SCID has a T-B- profile while NK cell numbers are variable. The abnormalities in T and B cell development have been attributed to a number of different mechanisms.¹⁶ Deficiency of ADA results in the accumulation of dAdo and deoxyadenosine triphosphate (dATP). Raised dAdo directly inactivates the enzyme S-adenosylhomocysteine hydrolase (SAH hydrolase)¹⁷ which is required for normal methylation reactions; dAdo has also been

Figure 3 Flow cytometric analysis of γ c expression in X-SCID patients. Peripheral blood mononuclear cells from a control sample (A) and two patients with T-B+ SCID (B, C) were stained with an antibody to common γ chain. An increase in fluorescence activity from isotype control is seen in (A), indicating normal γ chain expression. In (B) and (C) no γ chain expression is seen, thus confirming the diagnosis of X linked SCID.

reported to induce chromosome breakage and apoptosis in immature thymic lymphocytes. In addition raised dATP inhibits ribonucleotide reductase which is necessary for normal DNA synthesis. Because of the high turnover of lymphocytes, the highest amount of ADA expression is found in lymphoid tissue; this may explain the severe lymphotoxic effects of ADA deficiency.

Approximately 85–90% of ADA deficient patients present within the first year of life with severe clinical manifestations. However, there is considerable heterogeneity in the clinical and immunological phenotype with 15–20% of patients being diagnosed at 1–8 years of age.¹⁸ In some of these cases the pattern of infection is less severe, and this correlates with a less profound lymphocyte abnormality and degree of metabolic derangement. A few adults who were investigated for recurrent infections have also been shown to have a delayed onset ADA deficiency.¹⁹ Analysis of the ADA gene for mutations has shown a correlation between the mutation, amount of residual ADA activity of the mutant protein, and the clinical phenotype.²⁰ Diagnosis of ADA SCID can be made rapidly by analysis of dATP concentrations and ADA activity in washed red cells. In red cells of normal individuals, dATP concentrations are undetectable but are notably raised in patients with ADA deficiency. ADA catalytic activity in

patient erythrocytes is less than 1% of normal and is also significantly reduced in amniocytes or fibroblasts cultured from chorionic villus biopsy samples, thus allowing this assay to be used for prenatal diagnosis. Genetic analysis can also be used if a mutation has been identified.²¹

A rarer disorder of purine metabolism is purine nucleoside phosphorylase (PNP) deficiency.²² This enzyme, like ADA, is expressed in all tissues and is required to maintain the balance between production of dephosphorylated purines, detoxification to uric acid, and salvage back to the nucleotide concentration. Lack of PNP results in accumulation of a number of purine substrates, the most important being deoxyguanosine, which is phosphorylated to deoxyguanosine triphosphate (dGTP). Similar to dATP in ADA deficiency, dGTP exerts a lymphotoxic effect by inhibition of ribonucleotide reductase.²³ The immunological defects in PNP deficiency are variable but T cell function is most severely affected. Neurological defects are common and are mainly related to motor dysfunction. Cerebral palsy, spastic paresis, and ataxic diplegia have all been described.^{24–25} Diagnosis is based on analysis of enzyme activity and measurement of intracellular dGTP concentrations.

SCID caused by abnormalities in V(D)J recombination

The assembly of functional B and T cell receptor complexes is essential for the normal development of B and T lymphocytes. Both receptors consist of immunoglobulin or immunoglobulin like molecules, the diversity of which is generated through the process of V(D)J recombination. During this carefully regulated process, combinations of V (variable), D (diversity), and J (joining) genes are assembled to create unique sequences that code for specific receptor chains. The initial step in this process is the introduction of a DNA double strand break (dsb) at specific sequences that flank each receptor gene segment. This event is mediated by the action of two recombination activating genes, RAG1 and RAG2.²⁶ Subsequently, the double strand break is processed and modified gene segments are joined together. This latter process requires the action of a number of other molecules essential to the DNA dsb repair in all cells types: XRCC4, DNA ligase IV, Ku 70, Ku80, and the catalytic subunit of DNA dependent protein kinase (DNA-PKcs).²⁷ Murine models show that defects in any one of these molecules can give rise to a T-B- SCID phenotype.^{28–32}

In human SCID, a subgroup of T-B-NK+ patients have been shown to have defects in the RAG1 or RAG2 genes.³³ In an *in vitro* model, the mutant RAG proteins arising from these mutations were shown to be practically devoid of all V(D)J recombination activity, suggesting that in these patients recombination events cannot be initiated, and T and B cell receptor complexes cannot be assembled.

Mutations in the RAG genes have also been shown to give rise to Omenn's syndrome, a form of SCID which has a characteristic clinical

cal and immunological phenotype. Patients present with the characteristic infective complications of SCID, but in addition develop an erythrodermic rash with lymphadenopathy and hepatosplenomegaly (this clinical presentation can also be seen in SCID patients with engraftment of maternal lymphocytes). There is usually an associated eosinophilia and raised IgE but otherwise variable immunoglobulin concentrations. Circulating B lymphocytes are usually low or absent but T cells are detectable and display an activated phenotype. Detailed studies have shown that these T cells are oligoclonal and display restricted TCRV β usage.³⁴ In some families, children with both Omenn's syndrome and classical T-B- SCID have occurred, suggesting that the same gene defect can give rise to the different phenotypes.³⁵ Further analysis of Omenn's syndrome patients revealed that RAG gene mutations were responsible.³⁶ Mutant RAG proteins from Omenn's patients display residual V(D)J recombination activity, suggesting that assembly of some TCR complexes is possible and may explain why oligoclonal T cell populations are detected. At present definitive molecular diagnosis of T-B- SCID and Omenn's syndrome is dependent on the detection of RAG gene mutations. Routine protein expression analysis is not possible since the RAG genes are not expressed in mature cells found in peripheral blood, and lymphocyte precursors are in insufficient numbers in bone marrow. However, analysis of clonality in T and B cells in the peripheral circulation may give an indication of a defect in the V(D)J recombination process.

RAG defects are found in the majority of patients with T-B-NK+ SCID, but a significant number of T-B-NK+ SCID patients remain in whom no genetic defect has been identified. Some of these patients show increased sensitivity to ionising radiation, suggesting a defect in dsb repair mechanisms.³⁷⁻³⁸ However, analysis of the RAG genes and genes known to be involved in dsb repair has not detected any abnormalities, suggesting that other, as yet unidentified, gene defects may be responsible.

MHC class II deficiency (type 2 bare lymphocyte syndrome)

Major histocompatibility complex (MHC) class II deficiency is an autosomal recessive immunodeficiency syndrome resulting from defects in trans-acting factors essential for transcription of MHC class II genes; it is characterised by the presence of normal amounts of dysfunctional T and B cells (T+B+ SCID). All bone marrow derived cells in affected individuals fail to express MHC class II antigens (DR, DP, and DQ) and HLA-DM. In vitro studies have shown a specific defect in the binding of a protein complex, RFX, to the highly conserved X box of the MHC class II promoter in some of these patients.³⁹ Further studies in patients have revealed the presence of four genetic complementation groups (A, B, C, and D), reflecting the existence of four MHC class II regulators. Mutations have been found in subunits of the RFX complex in patients of

three complementation groups, RFX5 in group C,⁴⁰ RFXAP in group D,⁴¹ and most recently, RFX-B in group B.⁴² Group A patients do not display this defect and mutations have been found in another protein, CIITA, which acts as an MHC transactivator and is essential for the constitutive and inducible expression of all MHC class II genes.⁴³ Diagnosis is made by flow cytometric analysis of peripheral blood lymphocytes for the expression of MHC class II molecules. More detailed analysis of the genetic defect can then be undertaken.

Other genetic defects leading to SCID

The genetic abnormalities that lead to SCID in the majority of affected patients have been outlined. However, a number of other much rarer defects have been identified in a few patients. Abnormalities in components of the TCR receptor complex, CD3 ϵ and CD3 γ have been described in four patients to date.⁴⁴⁻⁴⁵ The phenotype in these patients was less severe than in classical SCID and an indication of the diagnosis can be gained from the mean fluorescence intensity of the TCR-CD3 complex on flow cytometric analysis. Other defects in TCR complex signalling can cause an SCID phenotype. Mutations in the ZAP-70 protein (which binds to the ζ chain of the CD3 complex) result in a selective CD8 lymphopenia.⁴⁶ It is thought that this molecule is essential for positive selection of CD8+ T cells during thymic maturation. A severe form of CD4+ lymphopenia has been shown to result from defective expression of the protein tyrosine kinase Lck which is involved in proximal TCR signalling.⁴⁷ Other individual SCID patients for whom novel molecular defects has been identified have also been reported.⁴⁸⁻⁴⁹

Strategies for diagnosis

Early clinical suspicion of susceptibility to infection is the first important step in making a diagnosis. Some reports have suggested that the absolute lymphocyte count found on most automated full blood count analyses may be a simple way to diagnose SCID.⁵⁰ However, while this may be useful in T-B- forms, it may be misleading in X-SCID and JAK-3 SCID where the lymphocyte count may be normal. In these and all other forms of SCID, analysis of lymphocyte subsets is necessary in order to detect the abnormalities in development of specific subpopulations. Following the algorithm in fig 1 can provide a useful guide to the molecular abnormality. Patients should then be investigated at specialist laboratories where the appropriate functional and genetic assays can be performed.

Summary

As we enter the post genome era, the genes responsible for many different types of SCID have been identified and it is very likely that many more will follow. The challenge remains to use this information to expand our understanding of the pathogenesis of the disease and to continue to develop improved methods of diagnosis and treatment for affected individuals.

A supraregional service for the molecular diagnosis of patients with SCID is provided by Great Ormond Street Hospital for Children NHS Trust. For referral and guidance regarding diagnosis of patients with suspected SCID or other severe primary immunodeficiency syndromes, please contact any one of the authors. Bone marrow transplantation for SCID and related disorders is also a supraregional service with two designated centres at Great Ormond Street Hospital for Children NHS Trust and Newcastle General Hospital.

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