SEVERE COMBINED IMMUNODEFICIENCY (SCID) – ADVANCES IN MOLECULAR DIAGNOSIS, NEONATAL SCREENING AND LONG-TERM MANAGEMENT

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ABSTRACT

Severe combined immunodeficiency (SCID) is a rare but important disorder. A true paediatric emergency, patients who are diagnosed early and referred appropriately have an excellent prognosis. Although SCID was first diagnosed only approximately 50 years ago, there has been a wealth of new knowledge gained in this relatively short period of time. Much has been learnt about the genetic basis of the disease, early diagnosis soon after birth and finally definitive treatment through immune reconstitution. An early haematopoietic stem cell transplant with an HLA-identical donor has a survival approaching 95% and there have been encouraging results using gene therapy in adenosine deaminase (ADA)-SCID and X-linked SCID.

INTRODUCTION

Severe combined immunodeficiency (SCID) is a group of rare single gene disorders with an incidence thought to be around 1:75 000 births.[1] This, however, may be an underestimate as some children with the disorder die from overwhelming infection prior to a diagnosis being confirmed. To date 10 different SCID phenotypes have been identified.[2] All types of SCID have a block in T-cell development with either direct or indirect impairment of B-cell immunity, making patients susceptible to infection by multiple pathogens. Patients with SCID who do not receive treatment in the form of immune reconstitution rarely survive beyond one year of life.[1,3,4]

CLASSIFICATION AND CLINICAL PRESENTATION

The various forms of SCID are classified according to their lymphocyte phenotype[5] (Table I). Knowledge of this immunological profile can be suggestive of the underlying genetic defect. Most cases of SCID have very low or absent T cells. Patients are then classified according to the presence (T^-B^+ SCID) or absence (T^-B^- SCID) of B lymphocytes and can be further classified by the presence or absence of natural killer (NK) cells.

Four different mechanisms have been identified as a cause of SCID:

1. *Premature cell death of lymphocyte precursors due to accumulation of purine metabolites*. This occurs in the autosomal recessive condition, adenosine deaminase (ADA) deficiency, which accounts for approximately 10-20% of all SCID cases.[2,6-8] Cell death occurs by apoptosis and all cell lines (T, B and NK cells) are affected. ADA is expressed in all tissues and thus also has effects on other organs such as the lungs, liver and brain.[9]
2. *Defective signalling through the common γ-chain-dependent cytokine receptors.* This is the most common form of SCID and accounts for >50% of all cases.[1,2,6] Interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, IL-21R all share a common subunit, the common γ-chain. A deficiency in either the function or expression of this chain results in the X-linked form of SCID (SCID-X1). Both mature T lymphocytes and NK cells

Table I. Classification of severe combined immunodeficiency

Lymphocyte phenotype		Inheritance	Chromosome
1. T^-B^- SCID			
• NK^+	RAG 1/2 deficiency	AR	11p13
	DCLRE1C (Artemis deficiency)	AR	10p13
• NK^-	Adenosine deaminase deficiency	AR	20q13.11
	Reticular dysgenesis	AR	
2. T^-B^+ SCID			
• NK^+	IL7α deficiency	AR	5p13
	CD45 deficiency	AR	
	CD3δ/CD3ε/CD3ζ deficiency	AR	
• NK^-	Common gamma-chain deficiency	X-linked	Xq13.1
	JAK3 deficiency	AR	19p13.1

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are absent. IL-7Rα gene mutation results in pure T-cell deficiency. B-cell development is normal in both these groups of SCID, despite the known role of IL-7 in B-cell survival and differentiation.[3,4] Janus-Kinase 3 (JAK-3) is a tyrosine kinase which binds to the γ-chain cytoplasmic region thereby mediating γ-chain signalling upon cytokine binding. A deficiency of JAK-3 results in a SCID phenotype identical to SCID-X1.

3. *Defective V(D)J (variable, domain, joining) gene rearrangement of T-cell receptors (TCR) and B-cell receptors (BCR)*. Approximately 30% of all SCID patients fall into this group.[2] Clonal diversity of T and B cells is generated by the somatic rearrangement of TCR and BCR. This is initiated by two recombination activating gene (RAG-1 and RAG-2) proteins. Mutations in the genes encoding these proteins result in faulty development of T and B cells, with sparing of NK cells ($T^-B^-NK^+$). Artemis is a protein involved in DNA repair after double-stranded cuts have been made by RAG-1 and RAG-2. A deficiency of this protein also results in impaired V(D)J arrangement and thus a $T^-B^-NK^+$ phenotype. Patients with artemesis deficiency also show increased sensitivity to ionising radiation.
4. *Defective pre-TCR and TCR signalling.* This rare form of SCID accounts for only 1-2% of all cases.[2] These pure T-cell deficiencies ($T^-B^-NK^+$) are a result of defects in key proteins involved in pre-TCR or TCR signalling such as CD45 phosphatase or a CD3 subunit (CD3δ and CD3ε).

Although we now know that SCID has many underlying genetic defects, all forms of SCID manifest with a similar clinical presentation, typically with severe infections early in life. The average age of presentation is 6 months, when maternal antibodies are declining.[3] Infections usually involve the respiratory tract or the gut and patients often present with persistent diarrhoea and failure to thrive. Opportunistic infections such as *Pneumocystis jerovici, Candida albicans*, and cytomegalovirus, as well as infections with adenovirus, respiratory syncytial virus and parainfluenza-3 also occur. Children who received BCG vaccination at birth are at risk of dissemination.[3,4] Maternal T-cell engraftment may occur in as many as 40% of children with SCID.[10] The maternal placenta is an incomplete barrier and maternal cells often occur in healthy neonates. In immunocompetent newborns these cells are cleared; however, as SCID infants lack T cells, maternal cells may persist. Clinical findings vary, with up to 60% asymptomatic and symptomatic graft-versus-host disease most commonly presenting with skin manifestations such as chronic eczematous skin rash or generalised exfoliative erythrodermia.[10]

Because of the severity of the clinical presentation, SCID should be regarded as a clinical emergency. Diagnosis should be confirmed as early as possible, as early diagnosis and management significantly improve the outcome.

PROSPECTS FOR NEONATAL DIAGNOSIS

Children who are diagnosed early and receive appropriate management and definitive treatment in the form of a haematopoetic stem-cell transplant (HSCT) have a far better prognosis than those children in whom the diagnosis has been delayed.[11] Prenatal diagnosis is now available for those parents who have a positive family history. However, many children with SCID are born to parents with no family history of the disease. Children with SCID are healthy at birth and have no external characteristics of the condition. The infectious complications which bring them to medical care may not initially be distinguishable from routine childhood infections. Thus diagnosis may be delayed.

Newborn screening would identify these children early, before infectious complications set in, thus giving them a better outcome after HSCT. In the USA a SCID Newborn Screening Working Group was convened in May 2007 with the goal of pursuing integrated approaches to SCID screening. SCID is thought to meet many of the accepted criteria for neonatal screening[12] in that it is fatal in infancy without definitive treatment, there is a short asymptomatic period after birth, effective treatment is available in the form of HSCT and more recently gene therapy,[13,14] early treatment improves outcome and the cellular and humoral deficiencies may be detectable through screening tests.

A simple and effective test still needs to be identified. Currently a number of screening test methods have been suggested for SCID (Table II), some of which are being implemented in pilot studies. SCID is a rare disease and therefore any screening test needs to have a high positive predictive value for it to be accepted for newborn screening. The main problem with currently proposed tests is the rate of false-positive or indeterminate results.[15] All proposed tests are based on the fact that all patients with SCID are unable to make normal numbers of T cells. However the test must also take into account that maternal T-cell engraftment may occur and patients with a T^-B^+ phenotype may have a normal number of B cells.

All children with SCID are lymphopenic at birth,[3,11,12] thus routine blood counts with manual differentials could diagnose nearly all cases of SCID at birth.[6] However there is a high number of both false-positives and -negatives, as not all children who are lymphopenic have SCID and some patients with SCID may have a low-normal absolute lymphocyte count because of the presence of B cells (IL2RG, JAK3 and IL7R gene defects) or maternal lymphocytes. Furthermore this test is potentially labour-intensive since it does not make use of the dried blood spots (DBS) collected rou-

Table II. Suggested screening test methods for SCID

Test method	Dried blood spot	Anticipated problems
FBC and absolute lymphocyte count	No	High false-positives and -negatives Labour intensive
Absent TREC	Yes	1.5% indeterminate results May need repeat test
IL-7 immunoassay	Yes	2-tier testing preliminary stages
Low CD3 immunoassay	Yes	Preliminary stages
Microarray mutation detection	Yes	Many gene mutations thus high false-negatives

FBC – full blood count, TREC – T-cell receptor excision circle

tinely from all neonates at birth in the USA. The UK Primary Immunodeficiency Network also uses a low lymphocyte count as an entry into their protocol for screening for SCID.[16] However a recent audit from Birmingham, UK,[17] found that although only 1 out of the 56 cases of lymphopenia was documented by the clinician, there were no clear missed cases of SCID. They therefore thought it would be more cost-effective to discuss cases of lymphopenia with an immunologist before doing further investigations for SCID.

T-cell receptor excision circles (TREC) are formed during normal thymic maturation of T cells. Chan and Puck[18] showed that children with SCID (and therefore a low number of mature T cells) have low or undetectable levels of TRECs, whereas normal healthy newborns have high levels. Furthermore adult T cells have around five times fewer TRECs; thus the TRECs found in neonatal DBS are unlikely to be derived from transferred maternal T cells and maternal T- cell engraftment is unlikely to interfere with the test. The TREC can be performed using real time PCR on DNA obtained from DBS on routinely obtained Guthrie cards. However, approximately 1.5% of anonymous Guthrie cards yielded indeterminate results because of failure to amplify TRECs. Further screening and possibly further diagnostic tests would be required in these cases. In 12 states in the USA a second Guthrie card is routinely requested for all infants in the first month of life. In these states a second test done on previous indeterminate samples would lead to a reduction of persistently indeterminate results in the range of 0.1% or less.[15] Pilot SCID screening using the TREC test will be implemented in Wisconsin within the next year.[15,19]

Immunoassays for IL-7 and T-cell-specific proteins are being evaluated as possible first-line or second-line newborn screening tests.[15,20] High IL-7 levels are associated with T-cell lymphopenic states and may possibly be useful in a two-tier system together with TREC testing as these tests combined give a specificity of 100%.[20] CD3 testing alone or in combination with CD45 or other T-cell proteins is another neonatal screening option with good sensitivity.[19] These two methods, however, are in preliminary stages of exploration.

Finally, direct detection of gene mutations using resequencing microarray chips is also an option. Although this potentially will have a high number of false-negatives, it would also give one an immediate specific gene diagnosis.

There is growing momentum for neonatal screening in the USA. However, in South Africa even if a suitable test is found, this is unlikely to be implemented in the near future. There is currently no universal neonatal screening for any disorder in South Africa. Perhaps as national prevention of mother-to-child transmission (PMTCT) screening of 6-week HIV-exposed infants improves, the infrastructure created by this screening will help with implementation of other important preventable conditions. Within the current constraints doctors seeing young infants should be aware of the possibility of the diagnosis in infants who present with severe opportunistic infections early on in life and investigate and refer timeously. Parents with a family history of SCID should also be offered early screening of subsequent offspring.

MANAGEMENT

There are three long-term management options for children with SCID. Firstly HSCT either through a matched sibling, a haploidentical parent or a matched unrelated donor. Children suffering from ADA-SCID have the further option of enzyme replacement therapy with pegylated ADA enzyme. Finally gene therapy has been an exciting new option although some caveats still seem pertinent.

Enzyme replacement therapy (ERT) for ADA-SCID

This has been used for ADA deficiency since 1987. PEG-ADA is given by intramuscular injection once or twice a week. This maintains a high ADA activity in the plasma (> 100 times normal) as there is no significant cellular uptake of PEG-ADA. Extracellular toxic metabolites deoxydenosine and adenosine are eliminated leading to normalisation of intracellular dATP levels. Over a 2-4-month period cellular and humoral immunity is reconstituted. Although many children recover full immunity in the short term, approximately half will require ongoing immunoglobulin replacement. In the long term some patients show a decline in T-cell numbers and become lymphopenic. Despite this, children seem to remain clinically well with normal growth parameters and free from infection.[8]

Haematopoietic stem cell transplant

HSCT from an HLA-identical sibling, if available, is the best option for a child diagnosed with SCID. Data from Europe between 1963 and 1999 showed a 3-year survival of 77%.[21] However, children receiving transplants after 1996 had a survival rate of over 90%, possibly because of improved severe infection management. What makes SCID such an attractive option for HSCT is the fact that because children with SCID have no T-cell function, they are unable to reject the graft and therefore do not need myeloablative therapy before transplant. The toxicity of the procedure is thus reduced, including problems such as neutropenia and mucositis, the need for platelet and red blood cell (RBC) transfusion, veno-occlusive disease and long-term impaired growth and sterility.[22] Graft-versus-host disease (GVHD) is also rare in these children.[21,22] An HLA-identical donor is available only in a minority of cases. Therefore most children will receive an HSCT from an HLA-mismatched related donor, made possible by new techniques which allow for T-cell depletion of the donor graft. However the prognosis for these children is significantly less than for their HLA-identical counterparts. Data from Europe looking at 294 patients showed a 3-year survival of only 54%; again this improved over time with children transplanted between 1996 and 1999 having a survival of 75%.[21] Data from Duke University in the USA also showed a 77% survival.[22] Improved survival is most likely due to better prevention of GVHD by more efficient methods of T-cell depletion and prevention and treatment of infection.[21]

There are three factors which play a role in the different survival rates between identical and haploidentical HSCT:[1]

- Although patients receive T-cell-depleted haploidentical HSCT some still experience GVHD which impacts on long-term survival.[21]
- Graft rejection is more common in patients with an NK^+ SCID phenotype. These children have a poorer prognosis as haploidentical HSCT is associated with an increased rate of failure of engraftment. This evidence suggests that NK cells have a role in allogeneic reactions in humans.[1]
- Kinetics of T-cell development is the major factor affecting different outcomes. In children receiving an HSCT from a related identical donor (RID) mature

T-cells become detectable 10-15 days post transplant.[1,23] These cells have a memory phenotype. T-cell counts reach normal values at 1-2 months post HSCT. These T cells are fully functional and provide sufficient immunity to protect patients. This however does not occur in haploidentical HSCT. Approximately 3-4 months post HSCT naïve T-cell TRECs appear in both groups suggesting neothymopoeisis.[1,23] Why this takes 3 months to develop is unknown. The thymus in patients with SCID develops abnormally, in the absence of T-cell precursors, and therefore may be unable to support thymopoeisis. However SCID patients receiving HSCT in the neonatal period have TREC-positive naïve T cells within 15-30 days and counts increase far more quickly, thus thymi of very young patients may not have been damaged by toxic metabolites (ADA-SCID) or prolonged absence of T-cell precursors (SCID-X1) or infections. Finding a way to speed up this immune reconstitution in haploidentical HSCT could potentially improve outcome.

Following most HSCTs, B cells remain those of the recipients,[1,23] indicating poor B-cell engraftment. B-cell function does not develop to the same extent as T-cell function and most patients require immunoglobulin infusions to prevent infections.[23] SCID-X1 and JAK-3 deficiency patients who lack donor B cells continue to have poor B-cell function while those with ADA-SCID and IL-7Rα deficiency have good host B-cell function. Pretransplant chemotherapy improves the B-cell engraftment in SCID-X1 and JAK-3 deficiency; however this does not guarantee B-cell engraftment and the risks associated with chemotherapy can outweigh the potential benefits.[23]

The final potential source for HSCT is HLA-matched unrelated donors (MUDs). A recent study compared outcomes of the three groups of HSCT,[24] They showed 80.5% survival in MUD HSCT. Patients receiving MUD HSCTs showed complete donor engraftment in 88.5% and only one patient still required intravenous immunoglobulin, suggesting good long-term immune reconstitution. With expanding donor bases worldwide MUD HSCTs may be an attractive alternative for patients without an HLA-identical sibling. Patients in this study all received transplants within 4 months of diagnosis and delay in finding a donor did not increase mortality prior to the HSCT. In fact, the authors felt that this provided time to stabilise the patient and improve nutritional status.

Much is known about the long-term survival of children who receive HSCT. However, not much is known about the long-term quality of life these patients experience after transplant. A recent study from Italy looked at clinical outcome 5 years after transplant.[25] They found that most children had attained satisfactory growth with only 17.5% below the 3rd centile for weight and 12.5% below the 3rd centile for height. All patients were attending school, although 3 patients required individualised support. Ten per cent of children had severe neurological problems. One of these children has ADA-SCID which is known to have a high incidence of neurological problems during long-term follow-up[9] because of the generalised metabolic abnormality associated with the condition. Viral infection around the time of HSCT may also influence clinical outcome. Most importantly the majority of patients from this study live at home and 60% do not require any treatment. This supports HSCT as an effective treatment for SCID providing not only long-term survival but also a good quality of life in most patients.

Gene therapy

This has been successful in treating ADA-SCID[26] and SCID-X1.[13,14] This was achieved by using retroviral vectors containing therapeutic genes to transduce patient $CD34^+$ cells. Patients with ADA-SCID receive a mild non-myeloablative chemotherapy prior to receiving the corrected stem cells to improve engraftment of the cells.[8] PEG-ADA, if started prior to gene therapy, is stopped in order to improve the selective advantage of the new cells[8,27] and has been associated with improved immunity following therapy. Follow-up of these patients has been short when compared with ERT and HSCT. Initial results are encouraging with good immune recovery and normal growth and development;[26] however long-term effects will have to be monitored.

Two groups in France[14] and the UK[13] have shown success using gene therapy in SCID-X1. This has been complicated by lymphoproliferative disorders in 4 children from the French group.[28,29] These seem to be related to retroviral vector integration close to the LMO2 proto-oncogene promoter, which was shown in the first 2 patients.[28] LMO2 induction by chromosomal translocations is known to be associated with a form of acute lymphoblastic lymphoma. Furthermore the initial 2 cases were 1 and 3 months of age respectively when they received gene therapy, suggesting that age may play a role in insertional mutagenesis. These patients received a higher dose of transformed cells and had rapid T-cell development early after gene therapy,[28] supporting a higher proliferative capacity of neonatal haematopoietic stem cells.[2] Both these factors may also have played a role in inducing mutagenesis.

No cases of insertional mutagenesis have been identified in the UK group. This may be due to slight differences in protocol. They did not add fetal calf serum to the cell culture medium, used a three times lower IL-3 concentration and used a different viral envelope[13,29] which may target a slightly different T-cell subset that is less prone to transformation.[29] However, follow-up in this group has been shorter.

The occurrence of insertional mutagenesis has uncovered the need to make gene therapy safer in the future. A number of methods are currently being investigated. Current retroviruses used in gene therapy have strong enhancer and promoter regions within the long terminal repeats (LTRs),[29] lentiviruses still have a preference for inserting near active genes but do not insert near the promoter or 5' regions of the gene.[30] The use of self-inactivating lentiviruses which lack LTR promoter activity after proviral integration may be a safer vector. The expression of the therapeutic gene would occur via an internal promoter with little enhancer activity; furthermore further safety may be achieved by using tissue-specific or gene-specific promoters to provide more tightly regulated gene expression.[2,27,29,30] Insulators are small DNA elements which act as barriers and therefore prevent promoter-enhancer elements and/or chromatin modifications from influencing the expression of neighbouring genes. These may also improve safety by limiting the activation of genes around the insertion site.[2,29,30] While this may decrease enhancer activity it will not eliminate it entirely. The insertion of a second transgene that encodes a prosuicide product such as herpes thymidine kinase would enable transduced cells to be killed by gancyclovir.[2,29,30] However there are unresolved problems using this approach such as expression and function of the suicide gene, the induction of *in vivo* resistance, the immunogenicity of the thymidine kinase and the limita-

tions to the use of antiviral drugs once the transduced cells have been injected.[29] Advances are expected from site-specific integration by targeting gene integration into neutral regions or sites that are known to be safe. This can be achieved by transferring integrases with rare integration sites into the human genome or by homologous recombination of small DNA fragments that can modify genomic DNA, thus simply repairing the mutation *in situ*.[2,29,30] This work is still in the preliminary stages.

Gene therapy has not been as successful in older children[31] despite effective gene transfer to $CD34^+$ cells. This suggests an age constraint to the efficacy of gene therapy, possibly because thymopoeisis may be influenced by infection, GVHD and physiological ageing.[31] Therefore, as in HSCT, immune reconstitution with gene therapy should be considered as early as possible.

CONCLUSION

Although first diagnosed only 50 years ago there has been remarkable advancement in the understanding of SCID resulting in improved survival and quality of life. SCID is a rare disease. It now has an excellent prognosis if treated early before the onset of severe infections. Implementation of neonatal screening may increase early diagnosis. Currently paediatricians should consider this diagnosis in any child who presents with severe opportunistic infections early in life and investigate appropriately.

Declaration of conflict of interest

The author declares no conflict of interest.

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