Narrative review of severe combined immunodeficiency—a purine metabolism disorder

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Background and Objectives: Severe combined immunodeficiency (SCID) is a primary immune deficiency that is a pediatric emergency. Left untreated, patients will die prior to 2 years of age from overwhelming infection. There are many known causes of SCID, but this review focuses on adenosine deaminase (ADA) deficiency.

Methods: An electronic search was performed on PubMed for relevant articles covering ADA deficient SCID including historical perspective, diagnosis, and treatment.

Key Content and Findings: ADA is a ubiquitous "housekeeping" enzyme, and its deficiency leads to buildup of toxic metabolites, which preferentially affects lymphocytes but also causes non-immune manifestations of disease. While previously diagnosed based on recurrent, severe, or unusual infection, SCID is now being diagnosed during its initial asymptomatic period thanks to recent implementation of T cell receptor excision circle (TREC) newborn screening, thus greatly improving survival of patients as treatment can occur before the onset of infection. To date, TREC screening has shown 100% sensitivity for SCID but also has brought to light other causes of T cell lymphopenia that were previously lesser known. ADA-deficient SCID can be treated through enzyme replacement therapy (ERT), hematopoietic stem cell transplantation (HSCT), or gene therapy, and advances continue to be made every day that improve cost-effectiveness and efficacy of these therapies and make them more widely available to patients.

Conclusions: This once-deadly disease is treatable and becoming more easily curable with the technological and scientific advances that allow for permanent immune reconstitution to become accessible to larger numbers of patients.

Keywords: Severe combined immunodeficiency (SCID); adenosine deaminase deficiency; gene therapy; pegademase bovine; hematopoietic stem cell transplantation (HSCT)

Introduction

Severe combined immunodeficiency (SCID) is a collection of at least 17 disorders characterized by defects of T cell number and/or function leading to severe impairment of the cellular and humoral immune response (see *Table 1*). This wide variety of genotypes under a common phenotypic umbrella is due to the complex interplay of steps that must occur for normal differentiation and maturation of lymphocytes. Even now there is still a significant minority of cases in which an underlying genetic mutation is not identified (1). Children with SCID whose immune systems are not reconstituted through hematopoietic stem cell transplantation (HSCT), gene therapy, or enzyme replacement (depending on genotype) die in infancy due to opportunistic and severe infections (2-6). This review will focus on SCID caused by adenosine deaminase (ADA) deficiency. ADA deficiency was the first molecular defect to be identified as a cause for immunodeficiency, identified by

Table 1 Known genes associated with severe combined immunodeficiency

Gene	Disease
ADA	ADA deficiency
AK2	Reticular dysgenesis
CD247	CD3ζ deficiency
CD3D	CD3δ deficiency
CD3E	CD3ε deficiency
CORO1A	Coronin-1A deficiency
DCLRE1C	Artemis deficiency
IL2RG	X-linked SCID (common gamma chain deficiency)
IL7R	IL7Rα deficiency
JAK3	JAK3 deficiency
LAT	LAT deficiency
LIG4	DNA ligase IV deficiency
NHEJ1	Cernunnos/XLF deficiency
PRKDC	DNA PKcs deficiency
PTPRC	CD45 deficiency
RAG1	RAG1 deficiency
RAG2	RAG2 deficiency

Dr. Robert Good in 1972 (7).

Methods

On 21 February, 2018, a search was conducted in PubMed with keywords "ADA deficient SCID," "severe combined immunodeficiency" "adenosine deaminase deficiency," "SCID screening," "ADA gene therapy," and "ADA SCID treatment". An additional search was performed 31 May, 2018 for any additional updates with the same search parameters. Only English articles were considered. Each author performed searches and chose relevant articles which were approved by all authors for inclusion in the references.

Metabolic pathway derangement

ADA is a 41 kDa soluble monomeric enzyme, made of 363 amino acids, that deaminates adenosine and 2'-deoxyadenosine (a product of DNA degradation generated during high cell turnover) to convert them to inosine and deoxyinosine, respectively, within the purine salvage pathway. It is found in the cytoplasm of erythrocytes and lymphocytes. It is encoded by the *ADA* gene, located on chromosome locus 20q13.11 (8). There is also a >200 kDa complex of ADA monomer bound to cell membrane glycoprotein CD26, found in activated T cells, epithelial cells, and medullary thymocytes, which has ectopeptidase activity and may also regulate certain cytokine and hormone activity (9,10). Lack of ADA function allows for buildup of adenosine and 2'-deoxyadenosine, which results in toxicity to lymphoid cells (especially in areas of high cell turnover such as the bone marrow and lymphoid tissues).

Excess 2'deoxyadnosine gets phosphorylated to 2'-deoxyadenosine triphosphate (dATP), which can exert proapoptotic effect by stabilizing the apoptosome complex, which then causes activation of p53, and can also activate apoptotic protease-activating factor 1. It can also inhibit ribonucleotide reductase, which prevents synthesis of new deoxynucleotides and therefore blocks DNA replication and repair. Additionally, it inhibits transmethylation, which is needed for lymphocyte activation, by inactivating S-adenosyl-L-homocysteine hydrolase (9,11-15).

Adenosine in elevated concentrations can activate the A2A adenosine receptor to suppress signals in activated immune cells, contributing to defective function in those T cells that do not undergo apoptosis (15).

ADA deficiency also seems to play a role in blocking T cell differentiation at early stages of development or in blocking proliferation of mature T cells, but studies have shown conflicting evidence (15). High levels of apoptosis occur in the typically-developing thymus as part of the process of negative selection of T cells that are strongly self-reactive, therefore high ADA levels are typically found in the thymus. ADA-deficient mice have decreased thymic and lymph node tissue volume when compared to healthy controls. There is a second form of ADA located in the extracellular compartment and encoded by the *CECR1* gene, but deficiency is mainly associated with vasculopathy (most notably polyarteritis nodosa) and only sometimes with a non-SCID immunodeficiency (15).

A spectrum of mutation severity allows for differing levels of residual ADA function and thus a spectrum of phenotypes, from severe immunodeficiency causing infections within the first months of life (about 90% of cases) to delayed onset presentations that are detected in early childhood due to gradual loss of T cell population over time, to healthy individuals with only minor deficiency of ADA (9,15,16). Over 70 mutations of the *ADA* gene have been identified and the disease is inherited in an autosomal

Table 2 Defining criteria for severe combined immunodeficiency

Severe combined immunodeficiency
<300 CD3 T cells/μL
<200 naïve CD4 T cells
<10% of normal T cell response to phytohemagglutinin
Or
Maternal T cells detected
Adenosine deaminase deficiency
Adenosine deaminase activity <1% of normal
Pathogenic mutations in both copies of *ADA* gene

recessive pattern. 60% of known mutations are missense (10). Other tissues can also variably be affected leading to lung, liver, and kidney damage, neurologic deficits including deafness and motor abnormalities, behavioral problems, and skeletal abnormalities (9,10,15-18). These non-immunologic problems are more prominent in ADA-deficient mouse models but their presence in knockout mice supports a metabolic cause for these derangements rather than as a complication of infection or treatment in these patients (9).

Patients with SCID on average have lower IQ than the general population, but patients with ADA-deficient SCID are lower on the scale than patients with other forms of SCID, with correlation to level of dATP at diagnosis. Behavioral problems can include hyperactivity, aggression, and attention deficit. Sensorineural hearing loss can be bilateral and affects over half of patients (19).

Lung abnormalities found in patients with ADA-deficient SCID include pulmonary alveolar proteinosis and other pulmonary inflammatory conditions such as pneumonitis. These can be fatal if not promptly treated (8,19).

Liver involvement is not typically severe in human patients (persistent mild elevations in liver enzymes), but this must be carefully monitored in the face of hepatotoxic chemotherapy regimens for HSCT conditioning or hepatotoxic antibiotics. In contrast, in ADA-deficient mouse models, hepatic disease is severe and often fatal (8).

Patients with ADA-deficient SCID can develop hemolytic-uremic syndrome, mesangial sclerosis, and cortical sclerosis of the adrenal glands (19).

ADA deficiency results in deficiency of T-cells, B-cells and NK cells (15). The only other form of SCID with similar phenotype is reticular dysgenesis. All other known forms of SCID have presence of B cells, NK cells, or both (16). The B cell defect is not thought to be due to impaired development in the bone marrow but rather impaired differentiation of immature B cells in the peripheral tissues due to defects intrinsic to them or due to lack of costimulatory T cell signaling, based on mouse models of ADA deficiency. B cells of patients with ADA-deficient SCID also have a decreased repertoire of antigen receptors due to effects of dATP accumulation on V(D)J recombination, a crucial component of antigen receptor diversity (15).

Epidemiology

SCID incidence was initially estimated at 1:100,000, but after the widespread implementation of newborn screening for the disease, the estimate has been updated to 1:60,000 in the US population, which is almost twice what was initially thought (1,20,21). ADA deficiency accounts for 11–20% of all cases of SCID and occurs in 1:200,000 live births, with a higher frequency in certain ethnic groups, thought to be due to founder mutation phenomena (1,15,16,22). The underlying genetic defect casing SCID can be identified only 80% of the time (16,23).

Clinical features

SCID has an initial asymptomatic period during which the patient has passive immunity via transplacental maternal IgG. Prior to availability of newborn screening for the condition, patients would present at 4–7 months of age with failure to thrive and recurrent and severe infections and diarrhea. Infants with a known family history of SCID were able to be diagnosed early through focused immune evaluation; however, 80% of patients have no known family history and therefore most patients were not identified until they were symptomatic (24,25).

Prior to initiation of widespread newborn screen, SCID was mainly characterized by severe and/or opportunistic infections. With more infants diagnosed prior to onset of infection, new criteria were put forth to define SCID based on laboratory parameters and to allow distinction from less severe phenotypes such as combined immunodeficiency and T cell lymphopenia (see *Table 2*).

Some patients with ADA-deficient SCID develop autoimmune disease, especially those with delayed onset phenotype or in patients who are starting to regain some immune function through enzyme replacement, thought to be due to disruption of typical tolerance-generating mechanisms of the immune system (10,15).

Table 3 Wilson and Jungner's criteria for screening for a disease

Criteria	Severe combined immunodeficiency
(I) Important health problem	Fatal within 1–2 years if untreated
(II) Accepted treatment for diagnosed patients	Hematopoietic stem cell transplantation
(III) Facilities available for diagnosis/treatment	Immunologists and transplantation facilities available worldwide
(IV) Recognizable in latent or early symptomatic stage	Immunologic abnormalities are detectable before onset of clinical symptoms such as infection or failure to thrive
(V) Screening test available	TREC[†] screen
(VI) Screening test is acceptable to the population	Uses existing system of dried blood spots used for other newborn screening tests which requires only a few drops of blood from heel prick
(VII) Natural history of the disease is understood	Recurrent, severe infections leading to failure to thrive and death
(VIII) Consensus on which patients should be treated	SCID[‡]-defining criteria established and protocols for hematopoietic stem cell transplantation guide when a patient can tolerate transplantation
(IX) Cost:benefit ratio balanced	TREC[†] screen is cost-effective
(X) Screening can be done on a continual basis	Part of ongoing newborn screening for all newborns

[†], T cell receptor excision circle; [‡], severe combined immunodeficiency.

Diagnostics

Historical screening approaches

Newborn screening for SCID fits Wilson and Jungner's principles of early screening for disease given the known natural history of the disease and availability of cost-effective confirmatory testing and treatment (26) (see *Table 3*). Patients who are identified and undergo immune reconstitution prior to the symptomatic period have a much higher survival rate than those who have had infections (27-32).

With the discovery of one molecular cause of SCID, scientists started looking for viable methods to screen for the disease. ADA and its metabolites can be measured in red blood cells and this was an early approach taken, but attempts to screen patients by measuring ADA levels in dried blood spots had high false positive and negative rates (25). For the general population, absolute lymphocyte count remained the most widely available screen for SCID for decades (33). However, this test lacks sensitivity and specificity for SCID and a threshold low enough to detect patients with high levels of B cells or circulating maternal lymphocytes would generate a high rate of false positives and it was not widely adopted (24,25,34,35). Routine flow cytometry to identify T cell subsets in cord blood was determined too time-consuming and expensive (24,25,35,36). Immunoassays to measure interleukin-7 (IL-7) (needed for proliferation/differentiation of immature thymocytes), CD3 (marker of T cells), and CD45 (marker of total leukocytes) have been proposed over the years but have not been made available on massive scale at reasonable cost.

Modern newborn screen

Current newborn screening for SCID uses quantitative polymerase chain reaction (PCR) to measure the number of T-cell receptor excision circles (TRECs) from the Guthrie dried blood spot. These are circular DNA fragments that are produced as a byproduct of normal T cell development in the thymus, proportional to the number of naïve T cells developing in the thymus (20,24,34,35). The number of TRECs is proportional to the number of T cells being produced in the thymus, and therefore can be used as a biomarker for T cell development (20).

TREC assay as a population-based screen was first proposed in 2005 (24) and subsequently implemented in the state of Wisconsin in a pilot study in 2008 (37). Over time, more and more states implemented the screening program and in 2018 it will be in place in every US state as well as the District of Columbia, Navajo Nation, and Puerto Rico (38). Worldwide, it has been implemented in at least 4 other countries and is soon to commence in at least 17 more across Europe, Asia, South America, and Oceana (35,39).

To date, there have been no documented cases of SCID missed by the TREC assay on newborn screening (40,41) and a recent systematic review of 13 case series

and prospective cohort studies on an international scale calculated test sensitivity of 100% (1).

Unfortunately, TREC-based screening does not identify cases of T cell dysfunction with normal T cell numbers, nor defects of T cell development that occur after the T cell receptor rearrangement, including ZAP70 deficiency, MHC class II deficiency, and delayed ADA deficiency, which disproportionately cause SCID in certain ethnic populations (22,35,40,42-48).

Immunologic & genetic evaluation

The TREC assay has been successfully identifying patients with SCID, but also patients with other causes of primary or secondary T cell lymphopenia. Therefore, further testing is essential to confirm results and correctly identify a patient's underlying diagnosis (18,20).

Patients with newborn screen positive for SCID are evaluated at SCID screening referral centers specific to each state. Management of the infant while confirmatory testing is performed can be on an outpatient or inpatient basis depending on TREC level, with patients having the lowest TREC values evaluated on an inpatient basis in order to keep them in protective isolation. Confirmatory testing includes complete blood count and flow cytometry to analyze lymphocyte subsets and often repeat TREC level. If, upon testing, a patient has ≥1,500 T cells/µL and ≥200 naïve CD4 T cells (those expressing CD45RA), then no further intervention is required. This happens in approximately 57% of cases of positive newborn screen. In about 16% of cases, patients have <300 T cells/µL or <200 naïve CD4 T cells on confirmatory testing and are hospitalized for protective isolation and further management of likely SCID or other severe immunodeficiency (18). Some centers only admit these patients if there is concern for active infection (40). Patients with an intermediate severity of phenotype (300–1,500 T cells/µL and presence of naïve CD4 T cells) must undergo further testing to characterize any other immune defects (proliferative responses to mitogens, quantitative immunoglobulin levels, and possible genetic testing) but can be managed inpatient versus outpatient on a case-by-case basis (18,40).

Patients with deficiency in T cells, B cells, and NK cells have the phenotype of ADA-deficient SCID, so measurement of ADA activity and metabolite levels should be obtained as they can establish the diagnosis faster than genetic testing. ADA activity <1% of normal is diagnostic of ADA deficiency (10,18). See *Table 2* for summary of diagnostic criteria. Chest x-ray can be considered to evaluate for presence of a thymus. HIV-1 antibody testing can be done to rule out vertically transmitted maternal HIV infection as a secondary cause for low T-cells (23,40). Screening evaluation for viral infections such as cytomegalovirus (CMV) is also important as part of potential pre-HSCT evaluation, along with HLA typing of the patient and family in order to identify hematopoietic stem cell (HSC) donors. The mother should be confirmed CMV negative before being allowed to breastfeed. If the patient has chimerism of maternal T cells, then that shows he or she may be able to accept HSCT with the mother as a donor without need for pre-transplant conditioning (18).

When performing such an array of testing in a small infant, one must be conscious of the volume of blood needed and take precautions so as not to induce iatrogenic anemia. A blood transfusion carries risk of causing graft-versus-host disease (GVHD) or transmission of CMV, so it is best avoided as much as possible. Using the absolute minimum required volume per test, prioritizing tests, and delaying some until a later time can help to minimize this risk (18).

If a particular cause of SCID is suspected based on phenotype, a particular gene may be sequenced. Otherwise, there are several commercially available genetic testing panels available for the known mutations that cause SCID (18). Newborn siblings of patients with ADA-deficient SCID should be screened with measurement of ADA activity or targeted gene sequencing if the patient's mutation is known. Carrier testing and prenatal testing for family members is also possible for families with an identified mutation (10).

Treatment/outcomes

Supportive therapies

Treatment for a patient with SCID includes immunoglobulin replacement therapy and antimicrobial prophylaxis against viral and fungal infections and *Pneumocystis jirovecii* with acyclovir, fluconazole, and trimethoprim-sulfamethoxazole, respectively (18,40). Live vaccines are contraindicated as they can lead to severe, fatal infections in patients with SCID. Inactivated vaccines are also typically held while a patient is on IVIG therapy as the donor antibodies offer passive immunity (40,49).

Patients with suspected SCID may not mount a typical immune response to infection, or may even be asymptomatic, so even minor signs of infection warrant

Table 4 Pearls for primary care providers

The TREC[†] newborn screen is 100% sensitive in detecting cases of classic SCID[‡] but also reveals other non-SCID[‡] causes of T cell lymphopenia
The TREC[†] newborn screen may miss cases of delayed onset ADA[§] deficiency, so patients with recurrent infections should be referred to an immunologist for evaluation
ADA§ deficiency causes a host of non-immunologic effects, including neurologic and behavioral problems, so close follow-up of development is required
Infants with SCID[‡] are at risk for failure to thrive, so growth must be carefully monitored
Even after undergoing immune reconstitution through enzyme replacement therapy, gene therapy, or hematopoietic stem cell transplantation, patients with ADA[§]-deficient SCID[‡] do not have a fully functional immune system and index of suspicion for infections must remain high, with prompt initiation of antibiotic therapy if indicated
Live vaccinations are contraindicated in patients with SCID[‡] unless the immune system has been fully reconstituted and should be cleared by the patient's immunologist
All vaccinations are held for a patient undergoing immunoglobulin replacement therapy, with the exception of seasonal influenza
Influenza and pertussis vaccinations must be kept up-to-date in family members of a patient with SCID[‡]
Infection transmission risk can be decreased at the primary care office by having an immunocompromised patient be the first morning appointment, thereby decreasing time in the waiting area exposed to other patients who may be ill
Diagnosis of infectious organism requires PCR[¶]- or culture-based methods rather than serologic methods in patients on immunoglobulin replacement therapy or patients with SCID[‡] whose immune system has not been fully reconstituted
Close communication with the immunologist regarding frequency of infections or adherence to medications can help best guide therapy for an individual patient

[†], T-cell receptor excision circle; [‡], severe combined immunodeficiency; [§], adenosine deaminase; [¶], polymerase chain reaction.

further evaluation with cultures and empiric treatment with antibiotics. Any viral testing will need to be via PCR rather than antibody serology (any present antibody will be maternal or from exogenous IVIG) (40).

Live vaccination of close family contacts of patients with SCID is controversial. While disease transmission is unlikely from live vaccination of close family contacts of the patient, some immunologists recommend against such vaccination except in cases of disease outbreak, though others disagree (40,49).

For patients being managed on an outpatient basis, infection control precautions must be taken by the family including to avoid crowded areas, daycares, and interactions with small children as well as strict handwashing and prevention of sick friends/family members from visiting. Visits to the primary care physician's office should be at the beginning of the day in order to minimize exposure to sick patients in the waiting room (40). See *Table 4* for recommendations to primary care providers.

If blood transfusion is needed, patients with SCID should receive leukoreduced, irradiated, CMV-negative packed red blood cells (18).

Immune reconstitution

Definitive treatment for ADA-deficient SCID is HSCT or gene therapy (3,5,25,28-31,40). Immune reconstitution prior to onset of infections is important for improved survival and decreased morbidity. In addition, early detection and treatment of ADA-deficient SCID in particular can prevent many of the non-immunologic manifestations of the disease that occur due to toxic accumulation of purines (9).

Enzyme replacement therapy (ERT)

Unique to ADA-deficient SCID is the option for ERT with polyethylene glycol-conjugated bovine ADA (PEG-ADA), which was first investigated in 1986 and FDA approved in 1990. It has been given to over 150 patients since its first availability (50). The conjugation to PEG allows it to circulate in the blood longer, and it is given as once or twice weekly IM injections (10,51). ERT can be a temporizing measure until more curative therapy is undertaken. It is frequently initiated in patients with ADA-deficient SCID

who do not have an HLA-matched sibling, as further search for a suitable HSC donor or consideration of gene therapy will take time. It is well-tolerated with a good safety profile, and can restore immune function [measured by T cell response to the mitogen phytohemagglutinin (PHA)] within 2–7 months of initiation, but has very short-term effectiveness once injections are stopped. Additionally, it does not provide complete immune reconstitution and some patients can develop immune dysregulation in the early phases of treatment. B cells of patients with ADA-deficient SCID who are on ERT show decreased proliferation *in vitro* and have low expression of B-cell activating factor receptor (BAFF-R), and only half of patients are able to discontinue IVIG (10,15,18,51). Many patients, while experiencing improvement in number of T, B, and NK cells, never reach normal levels, and even with maintenance of normal dATP levels in these patients, the numbers of T, B, and NK cells start to decline after 1–3 years of therapy for unknown reasons (10,51). One reason for incomplete immune reconstitution could be that PEG-ADA mainly exerts its effects in the extracellular compartment per murine studies, whereas it is most needed within lymphocytes (52). It is also costly over the long term ($200,000–$300,000 USD annually per patient) and with time, patients can develop IgG anti-ADA antibodies, which in a small subset of patients decrease the effectiveness of injections and eventually cause failure of therapy. This can be reversed in some patients through the use of immunomodulatory doses of intravenous immunoglobulin and corticosteroids, but benefits must be weighed against risks in using such regimens in immunocompromised patients (10,51,53,54).

Hematopoietic stem cell transplantation

HSCT is considered first-line treatment for all forms of SCID, including ADA-deficient SCID. The best outcomes in terms of immune reconstitution and transplant-related morbidity and mortality are in HSCT from matched sibling donors, but these are only available for less than 25% of patients (55). In addition to donor selection, timing of HSCT is critical. HSCT prior to 3.5 months of age (when maternal antibody is still at protective levels) results in a 93–96% survival rate as opposed to 50–82% in older infants with active or resolved infection at the time of transplantation (27-32)—a dramatic reversal of a previously universally fatal disease. The effect of age at transplantation is seen independent of the type of donor (related, unrelated, bone marrow, peripheral blood stem cells) (28). If HSCT

must be done at a time of concurrent infection without ability to treat the infection, it may be done without conditioning to decrease risk of transplant-related mortality. However, there is some evidence that engraftment in an unconditioned HSCT is of mature T cells with few prethymic progenitors, thus putting the patient at risk of eventual exhaustion of T cells without the ability for thymic renewal, as well as poor B or NK cell engraftment (28,56). If a conditioning regimen is used, many centers wait until 3–6 months of age to allow for adequate growth and development prior to transplantation in order to decrease risk of mortality (40). There is no consensus on the ideal conditioning regimen (none or immunosuppression, reduced intensity conditioning, or myeloablative conditioning) for patients with SCID, but studies are ongoing. Some patients require a second transplant or a boost of donor cells due to failure of engraftment with the first transplantation attempt (28).

If a patient with ADA-deficient SCID has been on ERT, and is planned to undergo HSCT without conditioning, the ERT must be discontinued to allow for the patient's T cell population to deplete again, or else risk GVHD (18).

A multi-center retrospective study of 106 patients with ADA-deficient SCID who underwent HSCT between 1981 and 2009 showed overall survival of 67%, higher in those who underwent HSCT from related donors (>80%), and notably most of those patients did not undergo conditioning prior to transplantation. Transplant-related mortality occurred in 20% of cases; more than half those deaths were due to pulmonary complications and/or sepsis. In this study, degree of HLA match appeared to play a bigger role in survival than conditioning regimen, and patients who underwent HSCT from related donors had faster T cell recovery and development of normal response to PHA in the first year, but by 2 years post-transplantation, immune recovery was similar between all groups. Use of PEG-ADA was not found to affect survival outcome (56).

Gene therapy

As stated earlier, HSCT is still considered first-line curative therapy for all forms of SCID. However, in patients with ADA-deficient SCID for whom a matched related donor is not readily available, gene therapy is an excellent alternative. It is fitting that the first immunodeficiency disease with a known molecular cause was also the first human disease to be treated with gene therapy, and now is the first disease with a commercially produced gene therapy vector (16,55).

In the United States, gene therapy is still considered experimental and clinical trials are ongoing. However, in 2016, the European Commission approved a commercial "advanced therapy medicinal product" consisting of an infusion of gene-corrected HSC prepared from a patient's own bone marrow-produced HSC, modified by a gamma-retroviral vector. This is the first *ex vivo* stem cell gene therapy in the world to receive such approval, but presently it is only available at a single hospital in Milan, Italy, due to short shelf life and need for administration by experts in gene therapy and HSCT (55). The first patient was treated with this product in March 2017 (57).

The advantage of gene therapy is that there is no risk of GVHD as cells are autologous.

Initial approaches to gene therapy relied on a gamma-retroviral vector to gene correct peripheral blood lymphocytes from a patient that were then re-infused over multiple treatments. Other concomitant studies used patient stem cells from bone marrow or umbilical cord blood instead. None of these early studies used a conditioning regimen. Newer protocols include reduced intensity conditioning in order to improve engraftment of progenitor cells in the bone marrow (55). Since 1990, more than 80 patients with ADA-deficient SCID have undergone gene therapy worldwide with 100% survival and, in most individuals, good reconstitution of immune function without need for ERT, though this may take 1–3 years from time of therapy (10,50,57,58).

Gene therapy has also been evaluated for X-linked SCID, but unfortunately some patients developed T-cell leukemia due to activation of proto-oncogenes (59,60). No cases of gene therapy-related malignancy have been reported in patients with ADA-deficient SCID, but because of concerns about possible insertional mutagenesis with the retroviral vector, research is ongoing with lentiviral vectors for gene therapy of both ADA-deficient and X-linked SCID (10).

Long-term monitoring

Patients who have successfully undergone immune reconstitution through one of the above methods should have at least annual follow-up to re-evaluate immune function (lymphocyte counts, quantitative immunoglobulin levels, response to mitogen stimulation) and to follow their neurologic development and growth. Additionally, several patients with ADA deficiency have been reported to develop dermatofibrosarcoma protuberans, a rare dermatologic malignancy, and should be monitored for development of this or other malignancies (10).

Future directions

Newborn screening

The method of TREC measurement continues to undergo improvement to increase cost- and time-efficiency (4,39). In some countries, TREC assay is done in conjunction with testing for ADA deficiency or purine nucleoside phosphorylase deficiency to more quickly identify the cause of SCID in screened patients (35). Other proposals to further refine sensitivity and specificity include combining TREC with IL-7 or CD3 protein assays, but these tests are still not available on massive scale at reasonable cost (25,34,39,61,62). Screening for other primary immune deficiencies is proposed by adding kappa recombining excision circles (KREC) testing to screen for B cell abnormalities or protein assays to screen for complement or granulocyte disorders (35). As genetic sequencing technology continues to improve in cost and time efficiency and more genetic defects causing SCID phenotype are identified, gene-based screening may become a viable option for SCID NBS as it is for cystic fibrosis (25,34).

Immune reconstitution

Current ERT is based on a bovine source of ADA, but studies are ongoing with recombinant human PEG-ADA (19). A recent Japanese study shows that ERT is possible through the use of gene-modified adipose cells, which can then be transplanted into a patient and secrete the desired protein. This could be explored as the basis for a more convenient method for ERT in the future, with a single procedure rather than weekly injections (63). Further studies are needed to determine why immune function declines after several years of ERT.

In cases where matched sibling donor or matched related donor are not available to a patient with ADA-deficient SCID, it remains to be determined whether gene therapy sooner or HSCT with unrelated donor after the delay it takes to secure a match would provide a better outcome. It remains unknown whether time to HSCT or degree of HLA match is the more important factor in graft performance in an uninfected infant.

More targeted conditioning regimens using monoclonal antibodies are being studied, especially for gene therapy, which could be used in patients who could not tolerate traditional conditioning (57).

As ongoing gene therapy trials for ADA-deficient SCID in the US generate more data, FDA approval for such therapy may be on the horizon, allowing even wider availability of this life-saving therapy. Additional trials are ongoing for lentivirus vectors, which also show promise for eventual commercial availability. The advantage over other vectors is their self-inactivation and higher efficiency of gene transfer, leading to higher levels of ADA expression (57). Other studies are evaluating new methods for cryopreserving the gene-corrected autologous cells to allow more centers to offer the therapy. Long-term outcomes data is being followed on all patients who have undergone gene therapy and will likely fuel further improvements in therapy to continue to make it more effective, cost-efficient, and accessible. Gene editing technology has also gained more attention in recent years and could potentially find applications for correction of ADA deficiency and other immunodeficiencies in the near future (64).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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