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Severe combined immunodeficiency. A model disease for molecular immunology and therapy

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Summary: Severe combined immunodeficiencies (SCIDs) consist of genetically determined arrest of T-cell differentiation. Ten different molecular defects have now been identified, which all lead to early death in the absence of therapy. Transplantation of allogeneic hematopoietic stem cells (HSCT) can restore T-cell development, thus saving the lives of SCID patients. In this review, the different characteristics of HSCT are discussed along with the available data regarding the long-term outcome. Transient thymopoiesis caused by an exhaustion of donor progenitor cells and possibly a progressive loss of thymus function can lead to a progressive decline in T-cell functions. The preliminary results of gene therapy show the correction of two SCID conditions. Based on the assumption that long-lasting pluripotent progenitor cells are transduced, these data suggest that gene therapy could overcome the long-term recurrence of the T-cell immunodeficiency. SCID is thus a disease model for experimental therapy in the hematopoietic system.

Introduction

Severe combined immunodeficiency (SCID) disorders consist of genetically determined blocks in the T-lymphocyte differentiation program. Overall incidence is estimated to 1 in 75 000 births. There is considerable genetic heterogeneity, as 10 different conditions all resulting in an SCID have been fully characterized (1) (*Fig.* 1). These conditions correspond to an intrinsic impairment of T-cell development, variably associated with defective differentiation of other hematopoietic cell lineages. In the absence of mature T cells, adaptive immunity is abrogated, resulting in a broad-spectrum susceptibility to multiple pathogens, among which a number of opportunistic microorganisms predominate (1–3). Patients with untreated SCID do not live beyond 6–12 months (2, 3). This outcome is why SCID was the first disease to be successfully treated by allogeneic hematopoietic stem cell transplantation (HSCT) (4) 36 years ago and was also the first to be successfully treated by gene transfer (5). SCID diseases represent

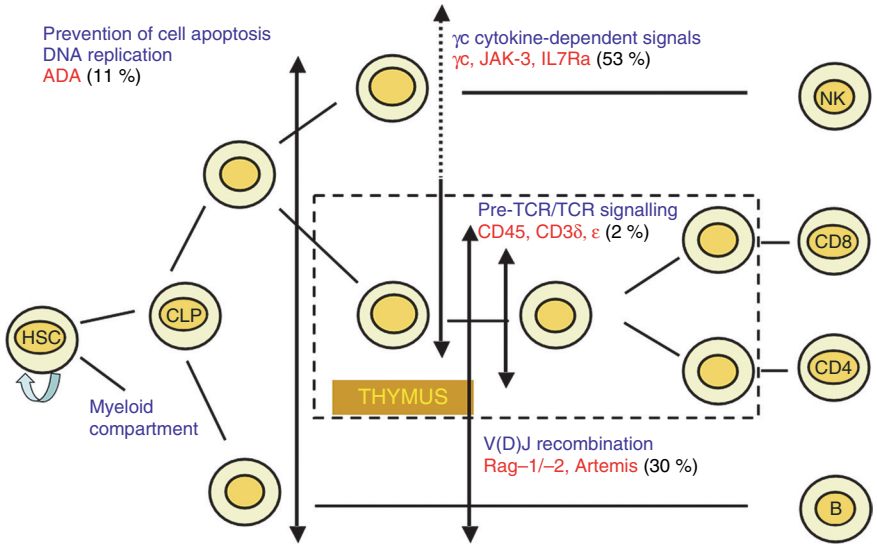

***Fig. 1.* Block in lymphopoiesis caused by severe combined immunodeficiency (SCID) conditions.** Vertical arrows indicate the approximate stages at which differentiation is impaired. HSC, hematopoietic stem cell (↻=self renewal); CLP, common lymphoid progenitor; ADA, adenosine deaminase deficiency. In brackets are the respective figures of given SCID condition frequency in our experience at Necker University Hospital in Paris. Dotted arrow indicates that natural killer (NK) cell differentiation is [γ-chain (γc), Janus kinase-3 (JAK-3)] or not (IL7-Rα) impaired in respective SCID conditions.

unique models for the analyses of these therapeutics, which have potentially much broader application.

Mechanisms of SCID (*Fig. 1*)

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

Premature lymphocyte precursor cell death as triggered by purine metabolism defect

Adenosine deaminase deficiency (ADA) results in an accumulation of adenosine, deoxyadenosine, and thereby deoxyadenosine triphosphate, which induce cell death by apoptosis, as lymphocyte precursors are exquisitely sensitive to their effects (6). A quasi absence of T, natural killer (NK), and B lymphocytes ensues when ADA gene mutations result in a complete absence of enzymatic activity.

Defective signaling through the common γ-chain-dependent cytokine receptors

Several cytokine receptors [interleukin-2 (IL-2), IL-4, IL-7, IL-9, IL-15, and IL-21R] share a common subunit, named the common γ-chain (γc) (7). The most frequent form of SCID, X-linked SCID (SCID-X1), is caused by mutations in the γc-encoding gene (8). It results in an absence of both mature T lymphocytes and NK lymphocytes. IL-7 interaction with its receptor (IL-7Rα/γc) induces survival, proliferation, and perhaps differentiation signals in lymphocyte precursors. It is unclear whether the signal is delivered to a common lymphocyte progenitor (CLP) or to committed lymphocyte precursors. IL-7 is required for T-cell development, because IL-7Rα gene mutations result in a pure T-cell deficiency (9). It is remarkable to observe that despite the known role of IL-7 in pro-B-cell survival/differentiation, B-cell development is entirely normal in γc and IL-7Rα-deficient patients (2, 3). NK cell deficiency in SCID-X1 is thought to result from the defective IL-15 interaction with its IL-15Rα/IL-2Rβ/γc receptor (7). γc signaling upon cytokine binding is mediated mostly by the Janus kinase-3 (JAK-3) kinase, a tyrosine kinase, which binds to γc cytoplasmic domain. Hence, JAK-3 gene mutations result in an SCID phenotype indistinguishable from SCID-X1 (10, 11), where inheritance is autosomal recessive.

Defective V(D)J recombination

A major step in T- and B-lymphocyte differentiation is the somatic rearrangement of the T-cell antigen receptor (TCR) and the B-cell antigen receptor (BCR), generating clonal diversity. This process is initiated by the recombination-activating gene-1 (Rag-1) and Rag-2 proteins, which cleave DNA at specific sequences surrounding the V, D, and J elements of *TCR* and *BCR* genes. Mutations of either *Rag-1* or *Rag-2* genes logically result in a faulty development of both T and B cells, whereas NK cell differentiation is spared (12). Hypomorphic mutation of either gene by enabling a limited generation of T-and B-cell clones does not result strictly in an SCID condition but rather in the Omenn syndrome, which is characterized by expansion of activated oligoclonal T cells (13). Another subset of $T^{-}B^{-}NK^{+}$ type of SCID is also the consequence of an impaired V(D)J recombination process, because one of the proteins involved in the non-homologous end joining repair pathway (NHEJ), Artemis, is defective (14). In this case, V(D)J rearrangement is impaired, because the hairpins that join DNA

strands of coding elements and have been formed by the Rag-1/2 complex cannot be resolved. Another defect in NHEJ has been found, DNA ligase-4 deficiency, but the hypomorphic mutations so far described all lead to a partial T + B-cell immunodeficiency, thus not strictly an SCID phenotype (15).

Defective pre-TCR/TCR signaling

Rare cases of SCID consisting of pure T-cell deficiencies have been attributed to defects in key proteins involved in pre-TCR/TCR signaling. Deficiency in the CD45 phosphatase has been reported in two cases of SCID (16, 17), while defects in CD3δ and CD3ε (18) have also been described. In these cases, failure of T-cell development likely results from defective signaling through the ptα/TCR-β pre-TCR and/or the TCR at the positive selection stage. It is remarkable to observe that a complete deficiency in CD3γ, another subunit of the pre-TCR/TCR signaling complex, leads to a milder immunodeficiency (19), showing that the CD3 subunits exert different functions.

Other defects in T-lymphocyte precursor signaling have been described, such as ζ-associated protein of 70 kDa (ZAP-70) deficiency or human leukocyte antigen (HLA) class II gene expression deficiency, but these mutations do not fully abrogate T-cell differentiation. They are thus not classified as SCID.

In our experience, SCID conditions caused by these four mechanisms account for 96% of studied SCID cases (in a cohort of 149 patients referred to our center at Necker University Hospital, Paris). Residual cases consist of the rare, ill-defined reticular dysgenesis (RD) condition (20). RD is characterized by profoundly, but not completely, defective lymphocyte differentiation associated with broadly impaired hematopoiesis resulting in agranulocytosis and in some cases thrombocytopenia. Some of the RD patients, in addition, have perception deafness (20). The mechanism of RD is unknown, but it likely is a complex hematopoietic defect. In addition, there are still a few more uncharacterized pure T-cell deficiency cases.

Hematopoietic stem cell transplantation, a life-saving procedure in SCID

Injection of bone marrow cells to SCID patients can correct the immunodeficiency well enough to save patients' lives (4). Hundreds of patients have benefited worldwide from this procedure in the last 35 years (21, 22). Therefore, HSCT is legitimately considered an emergency treatment. The unique particularity of HSCT in SCID is that there is no requirement for a myeloablative therapy to enable donor cell engraftment. This situation brings about a significant clinical advantage, as toxicity of the procedure can be much reduced. In addition, occurrence of graft versus host disease (GVHD) following HSCT from an HLA-identical sibling is extremely infrequent, for reasons which remain unclear (21, 22). This favorable setting also led to the development of haploidentical parental HSCT for the many SCID patients who do not have an HLA-identical sibling (23), in the early 1980s. This development was made possible by the availability of techniques allowing for depletion of mature T cells from graft inoculum.

The overall survival rate of SCID patients who underwent an HSCT from an HLA-identical sibling is good, being over 80% in patients treated from 1968 until now (21, 22). With the advance of severe infection management, the survival rate is over 90% since 1996 (22). Survival rate of patients treated by a haploidentical T-cell-depleted HSCT is not as good, with a long-term survival rate ranging from 50 to 78% (21, 22). Again, results have improved over time (from 35% survival in patients transplanted before 1985 in Europe to 75% in those treated between 1996 and 1999) (22). Better prevention of GVHD and treatment of infections likely account for the improved survival. But, survival rates still do significantly differ between SCID patients treated by HLA-identical or haploidentical HSCT, with the exception of patients treated early after birth and free of infections (21). Three parameters play a role in determining survival: GVHD, graft rejection, and kinetics of T-cell development.

Despite the reduction of mature donor T-cell infusion to very low numbers by using either E-rosetting/soy bean agglutinin selection (21) or $CD34^+$ cell selection (24), there is a residual incidence of severe acute/chronic GVHD in SCID patients treated by haploidentical HSCT, which impacts survival (22). Graft rejection appears to be an issue for patients with the NK^+ type of SCID, because haploidentical HSCT is associated with an increased rate of failure of engraftment and a poorer prognosis for patients with this SCID subset (25). In a European survey published in 1999 (25), it was shown that only 35% of NK^+B^- SCID patients were long-term survivors versus 60% of NK^-B^+ SCID patients. These results are the strongest evidence for a role in NK cells in allogeneic reactions in humans. The third and major parameter affecting outcome relates to the different pattern of T-cell reconstitution after either HLA-identical or haploidentical marrow HSCT.

Kinetics of T-cell development after HSCT

HLA-identical HSCT consists of the injection of the whole bone marrow cells, i.e. progenitors as well as mature cells including a number of T cells (originating from the marrow or the contaminating blood), whereas only selected hematopoietic progenitors from haploidentical donor are injected. This procedure results in a very different pattern of T-cell reconstitution (26) (*Fig.* 2). Mature T cells become detectable in patients' blood as early as 10–15 days after HLA-identical HSCT. These T cells essentially have a memory phenotype. T-cell counts reach normal values within 1–2 months. These T cells are fully functional and provide sufficient immunity to protect the patients. No such T cells are seen in recipients of HLA haploidentical HSCT. Naïve T cells with T-cell receptor excision circles (TRECs) appear in the blood only after 3–4 months after either HLA-identical or haploidentical HSCT (26). Therefore, T-cell reconstitution is bimodal: (i) early expansion of mature T cells resulting from both homeostatic and antigen-driven expansion and (ii) neothymopoiesis in host thymus leading to the late appearance of mature naïve T cells.

Antigen-driven expansion of mature T cells following HLA-identical HSCT can be dramatic. This finding is exemplified by the occurrence of an abrupt wave of donor-derived cytolytic $CD8^+$ T cells specific to maternal alloantigens early (approximately 10 days) after HSCT in SCID patients with maternal T-cell engraftment (27). It is estimated that the division time of those alloreactive clones is on the order of 6 h. Such antigen-driven expansion has also been observed following HLA-identical HSCT in SCID patients with ongoing cytomegalovirus infection (unpublished data), indicating the usefulness of such brutal T-cell responses.

What remains unclear is why there is a need for a 3–4 month period to observe effective neothymopoiesis after HSCT in SCID patients. *In vitro*, human T-cell maturation in fetal thymic organ cultures can be observed within 25–30 days (28).

The thymic immaturity in SCID, characterized by the absence of corticomedullary differentiation and the absence of detectable dendritic cells (29), can account for this observation. It is known that interaction between T-cell precursors and thymic epithelial cells are required in order to induce thymic epithelial cell maturation. It is also possible that this 3–4-month period is also required to achieve a measurable post-thymic expansion. Nevertheless, it is striking to observe that T-cell development in HSCT performed in settings other than SCID is not more rapid. This finding strongly suggests that there is an incompressible period of time required to achieve efficient T-cell production in humans. The only parameter that has been shown to influence the kinetics of neothymopoiesis is the age of the recipient. In SCID patients younger than 3 months, detection of $TREC^+$ naïve T cells after haploidentical HSCT or gene therapy (for SCID-X1) occurs slightly earlier (by 15–30 days) and the increase in cell counts is much faster (30, 31). It is possible that the potential of a young thymus to support lymphopoiesis is intrinsically higher. Alternatively, as compared to thymi from older patients with SCID, thymi from very young patients have not yet been injured either by direct consequences of the genetic defect, as in ADA deficiency, or by the indirect consequences of a prolonged absence of T-cell precursors as in SCID-X1 (29) and/or of infections (+/–GVHD).

Given the major clinical consequences of the prolonged T-cell immunodeficiency in post-haploidentical HSCT in SCID patients, it would be of crucial importance to find a safe way to speed up immune reconstitution or alleviate the immunodeficiency. Several possibilities do exist, but none has yet proven to be both efficient and safe.

It is tempting to consider the use of cytokines such as IL-7 (and perhaps IL-15), which have been described in experimental transplantation conditions to induce more rapid T-cell differentiation and to increase homeostatic expansion (32). However, we found a mild effect only, with use of low cell dose of HSCT (33). In addition, there are no clinical data

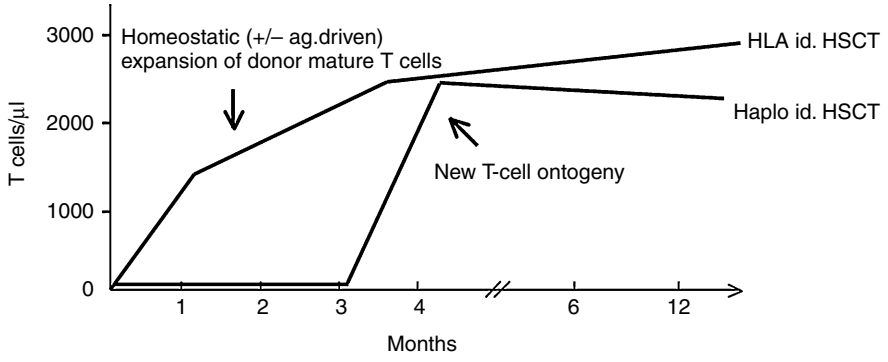

***Fig.* 2. Kinetics of T-cell development after hematopoietic stem cell transplantation.** Curves are indicative, not real data. HLA id HSCT, HLA-identical hematopoietic stem cell transplantation; haplo id HSCT, HLA haploidentical hematopoietic stem cell transplantation; ag, antigen.

available yet. Usage of keratinocyte growth factor with trophic effects on the thymus is another option, but it remains to be tested. We likely need to better understand the physiology of thymic T-cell maturation before any kind of manipulation can be seriously considered (28). Another option to consider is the reconstitution of a mature T-cell pool by the injection of mature T cells either *in vitro* or potentially *in vivo* devoid of alloreactivity (34–37). The technology(ies) is(are) however, not yet fully mastered to avoid the risk of residual GVHD, while a sufficient number of competent T cells can be infused.

Tolerance and education

In the early 1980s, haploidentical HSCTs were performed in SCID patients based on the assumption that haploidentical T-cell precursors will be educated into host thymus to be self-tolerant and able to recognize antigens on host dendritic cells. Previous experimental work in the mouse indicated that this outcome was likely to happen.

Several studies have verified that after haploidentical HSCT in SCID, donor-derived T cells were not activated by unshared host HLA alloantigens (38, 39). The mechanism for this lack of reactivity (deletion, anergy, etc.) has not been determined. In parallel, it has been found that donor-derived antigen-specific T cells after haploidentical HSCT in SCID patients were able to recognize antigen (tetanus toxoid and influenza virus peptides) in the context of host HLA class II molecules (both shared and not shared with the donor) as well as donor HLA class II molecules not shared with the recipient (40 and unpublished data). These data show that positive selection likely can be driven in humans by epithelial cells, as previously shown in the mouse, but also may be driven by donor antigen-presenting cells that have colonized the host thymus. It was not possible however to exclude the possibility that a few antigen-specific donor memory T cells were transferred with the graft, accounting for donor-restricted responses. Nevertheless, the recent demonstration that host T cells can recognize antigens presented by host antigen-presenting cells after fully HLA-incompatible thymus transplantation in patients with Di George syndrome shows that host antigen-presenting cells colonizing the thymus can mediate positive selection (41). This 'rupture with the dogma' has been further confirmed in an animal experimental setting of tetraparental chimeric mice (42). In any case, these results confirm that haploidentical HSCT in SCID can lead to the development of fully functional T cells in a new environment.

How many progenitors are required to reconstitute T-cell immunity?

How many stem cells or T-cell progenitors are required to efficiently reconstitute protective T-cell immunity after treatment is unknown. Two pieces of data do however provide useful information suggesting that very few cells are required, because their ability to divide prior to the stage of TCR gene rearrangements, i.e. clonal diversity generation, is high. Partial spontaneous reversion of T-cell immunodeficiencies has now been observed for four diseases: ADA deficiency (43), SCID-X1 (44, 45), Wiskott–Aldrich syndrome (46), and NEMO deficiency associated with anhydrotic ectodermal dysplasia and immunodeficiency (47). These results collectively demonstrate that one or a few T-cell precursors with full potential for development can give rise to a recognizable and persistent pool of functional, mature T cells.

A reverting mutation of γc in an SCID-X1 patient led to the development of a blood T-cell pool that was reduced by half compared to age-matched controls (44). Antigen-specific responses, for instance to varicella-zoster virus (VZV), could be documented. Of note, all T cells had a memory phenotype compatible with a single event of T-cell maturation followed by peripheral, presumably homeostatic driven expansion (44). In-depth analysis of the TCR-β repertoire of peripheral T cells of this patient by the immunoscope method led to the estimation that approximately 1000 distinct TCR-β-expressing clones were present (45), accounting for 1% of the size of a control memory repertoire (48). This remarkable finding indicates that a minimal estimation of precursor cell expansion before the onset of TCR-gene rearrangements was 10–11 division cycles. This observation also clearly indicates that such proliferation is γc-dependent, presumably through interaction of IL-7 with its receptor (discussed above). In summary, one precursor can generate approximately 1% of the T-cell repertoire. Such cells can expand and persist over years (they were detected for 5 years in this child before he underwent transplantation due to a decline in immune functions) (45).

Confirmation of these findings has been provided by the analysis of the clonality, as determined by retrovirus integration sites, after gene therapy of SCID-X1 (γc deficiency) (5, 31, 49). Integration of provirus at a given position of the genome of precursor cells can be regarded as a clonal signature, as all progeny cells carry the same integration position of the provirus. Therefore, a comprehensive analysis of the number of different integration sites found in T cells after gene therapy is telling us how many precursor cells were transduced and gave rise to detectable T cells. The linear

amplified-mediated polymerase chain reaction (PCR) method (50), based on the principle of a ligated-mediated PCR, has enabled the performance of this analysis. It has been found that the number of different clones as defined by provirus integration sites in T cells is on the order of the hundreds, while T cells were found highly polyclonal (31, 49) (discussed below). This result confirms the very high γc-dependent proliferative potential of precursor T cells. It has been also shown that T cells derived from a single clone, with a somewhat polyclonal pattern of TCRs, could persist after gene therapy for ADA deficiency (51). The concept appears therefore valid for different forms of SCID. It is possible that there are also not so many precursors involved in T-cell differentiation after HSCT performed in SCID patients. One can even speculate that this process does not differ greatly from physiological T-cell development.

What is the nature of the progenitors that reconstitute the T-cell pool after HSCT?

In the absence of a myeloablative conditioning regimen, there is a split chimerism in most SCID patients (>80%), with T cells and NK cells being of donor origin in patients with a T^-NK^- type of SCID (52) (*Fig.* 3). All other leukocyte subsets as well as hematopoietic lineages are of host origin. This finding is true after both HLA-identical and haploidentical HSCT (*Fig.* 3). This chimerism pattern is very unusual compared to classical settings of HSCT. Together with the quasi absence of donor cells in the bone marrow (apart from T cells) (53 and unpublished data), these data indicate that hematopoiesis remains of host origin, despite the HSCT.

The model depicted as an example for NK^-B^+ SCID in *Fig.* 4 can be proposed: T-cell progenitors, committed pro-T cells, CLPs, multipotent progenitors, or hematopoietic stem cells (HSCs) of donor origin migrate to and colonize the thymus early after HSCT. A wave of T-cell differentiation ensues, which is sufficient to (re)populate all T-lymphocyte niches and account for the development of efficient T-cell immune responses (26, 54, 55). There is however no engraftment of stem cells in the bone marrow. The prediction of this model is that after several years, the potential for T-cell lymphopoiesis is exhausted, leaving the patient with a given set of T cells for the remainder of his/her life.

It is striking to observe that NK cell reconstitution is not as good as T-cell reconstitution. This observation has been made both following HSCT and gene therapy, suggesting that the capacity for expansion of NK cell precursors is much more reduced compared to T cells (5, 21). It is presently unknown whether incomplete NK cell reconstitution might have clinical consequences. By analyzing clinical manifestations of SCID patients, transplanted more than 10 years ago, the only significant difference observed between patients who originally had an NK^- SCID and those who had an NK^+ SCID is the more frequent occurrence of chronic skin human papilloma virus (HPV) disease (56). NK^- SCID patients exhibit a significantly lower NK cell count (median 45/μl) than the NK^+ SCID patients, 10 years or more after HSCT (median 178/μl). However, within the former group, patients with or without chronic HPV disease do not differ for NK cell counts and function (56).

Another consequence of the lack of donor stem cell engraftment is the usual absence of donor B cells (*Fig.* 3). This finding shows that B-cell precursors contained in bone marrow inoculum have a limited capacity for differentiation into multiple B-cell clones and potentially that B cells have a limited half-life. It is also possible that competition with the host B-cell lineage in B^+ SCID or even pro-B cells in B^- SCID impairs donor B-cell differentiation. The frequently defective donor B-cell

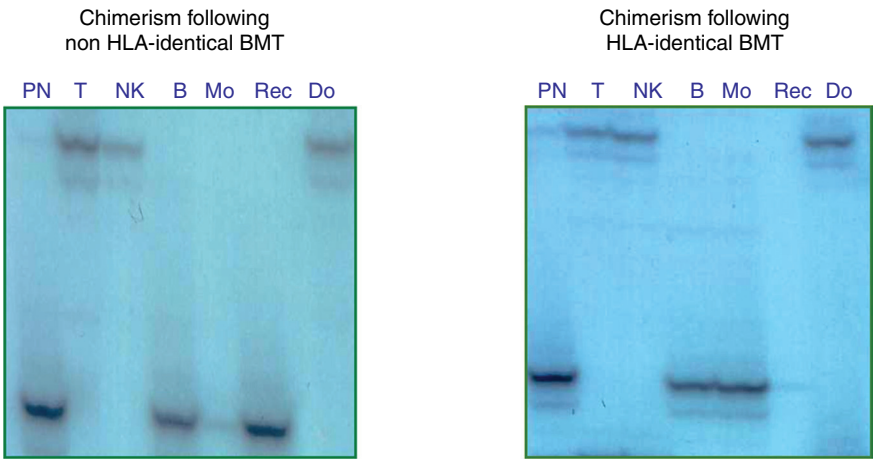

***Fig.* 3. Split chimerism after hematopoietic stem cell transplantation in NK^-B^+ severe combined immunodeficiency.** Two examples of chimerism are shown for the different leukocyte subsets that were separated by fluorescence-assisted cell sorting. Differently migrating bands depict DNA polymorphic microsatellite markers. PN, polymorphonuclear leukocytes; T, T lymphocytes; NK, NK lymphocytes; B, B lymphocytes; Mo, monocytes; Rec, recipient; Do, donor.

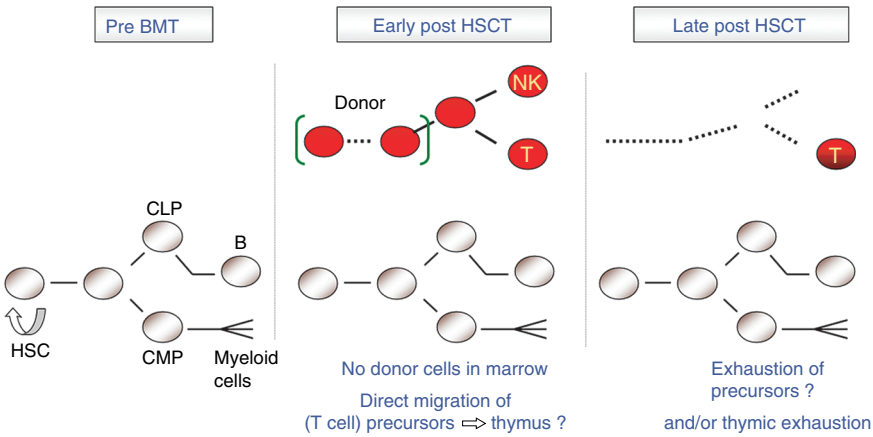

Fig. 4. Model of hemato-lymphopoiesis pre and post hematopoietic stem cell transplantation in NK$^-$B$^+$ severe combined immunodeficiency. HSC, hematopoietic stem cell; CLP, common lymphoid progenitor; CMP, common myeloid precursor.

reconstitution therefore leads to persistent B-cell deficiency in a majority of patients (21, 54). These patients require immunoglobulin substitution. This need is explained by either an absence of B cells (B$^-$ SCID) or the presence of defective B cells (B$^+$ SCID caused by γc or JAK-3 mutations). Although in the latter case, some B-cell function can be preserved (52, 57), 80% or so of patients do require intravenous immunoglobulin substitution. A minority of SCID patients has long-lasting functional B-cell immunity associated with host B cells, and they are mostly patients who had an SCID with normal B cells (IL-7Rα and CD3 deficiencies).

Consequences of the absence of stem cell engraftment on long-term T-cell immunity

The prediction of the above-mentioned model (*Fig.* 4) is that there should be a gradual decay in newly formed T cells after HSCT in SCID patients performed without myeloablative therapy. This finding was first shown by Patel *et al.* (58), by quantifying TRECs in circulating T cells sequentially after HSCT. Presence of TRECs denotes that T cells did not undergo division following TCR gene rearrangement and thus detects naïve T cells. Such TREC$^+$ T cells are no longer detectable 10–12 years after HSCT (58). This figure does not tell us when exactly thymopoiesis ceases, as naïve T cells can persist for some time, but these results confirm that these patients do dispose of a finite set of T cells in the absence of further immune intervention.

There is an alternative explanation to this unequivocal fall in naïve T-cell counts: the thymus for SCID patients, as discussed below, is not able to support thymopoiesis as well as healthy thymus, because its development was initially abnormal in the absence of functional T-cell precursors. In addition, thymi in SCID patients might have been further damaged by infectious events or GVHD occurrence (29). Both hypotheses are not mutually exclusive but bear distinct consequences in terms of immune intervention (discussed below). The failure in our experience of secondary haploidentical HSCT to improve T-cell immunity, when performed several years after the first HSCT, is consistent with the latter hypothesis (unpublished data).

In order to further assess the significance of TREC$^+$ T-cell detection after HSCT in SCID patients, we have attempted to correlate quantitation of TREC$^+$ T cells with chimerism. Within the group of patients who received a haploidentical HSCT more than 10 years ago, there is a very strong correlation between detection of TREC$^+$ T cells and donor myeloid chimerism. Indeed, virtually no TREC$^+$ T cells were detected in patients with no myeloid donor chimerism, whereas TREC$^+$ T cells were found, albeit in variable numbers, in patients with evidence for donor myeloid cells (S. Hacein-Bey *et al.*, unpublished data). These data, though preliminary, strongly support the concept that when donor myeloid cells are present, as a marker of donor-derived hematopoiesis, thymopoiesis does persist, even if the thymus is not entirely normal. Hence, perhaps thymic function in SCID patients is lost when donor progenitor cells, which have emigrated to it, are exhausted. If true, this concept implies that everything possible should be done to maintain uninterrupted thymopoiesis in treated SCID patients (discussed below).

Immunological and clinical consequences of the decline in thymopoiesis after HSCT in SCID patients are far from being negligible (*Fig.* 5, *Table* 1). As shown in a recent survey of the European registry, at last follow up, 16% of SCID patients transplanted with HSCT from an HLA-identical donor and 18% of SCID patients transplanted with HSCT from a parent had a T-cell immunodeficiency [defined as T-cell lymphocytopenia (<1000/μl) or defective *in vitro* antigen T-cell proliferation] (22).

There are other factors in addition to loss of thymopoiesis that can affect long-term T-cell immune reconstitution:

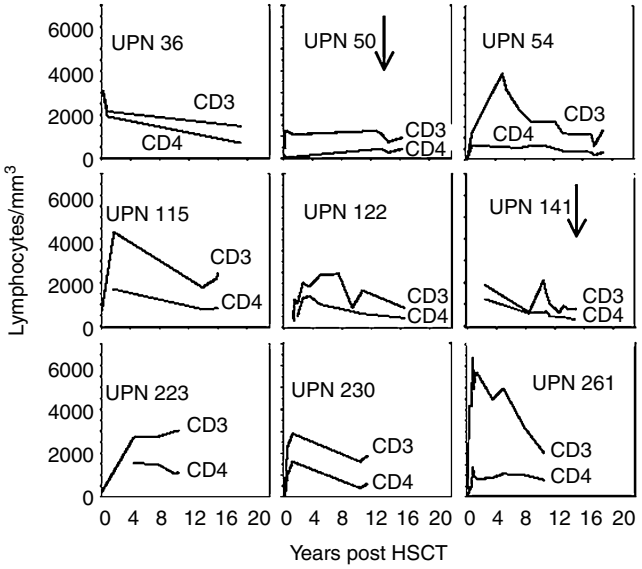

***Fig. 5.* Examples of T-cell count evolution over time after hematopoietic stem cell transplantation (HSCT) in nine patients with severe combined immunodeficiency.** CD3 and CD4 absolute counts are depicted for each patient. UPN, unique patient number. Arrow (UPN 50 and 141) indicates boost of HSCT.

occurrence of a GVHD (54), likely affecting thymus function (22, 25). As expected, it has also been shown that there is a strong correlation between detection of $TREC^+$ T cells and TCR repertoire diversity (30). All of these indications point to a progression of the immunodeficiency over time in transplanted SCID patients in the absence of donor stem cell engraftment.

How can long-term immunity be improved in SCID patients?

There is certainly a large variability in the potential long-term consequences of declining T-cell immunity in SCID patients. Patient follow-up in the next 20–30 years will define to what extent this will become a serious clinical issue. Declining T-cell immunity nevertheless emphasizes the need for long-term careful follow-up of these patients. Of note, a patient transplanted in 1975 has developed a gastric EBV^- (Epstein Barr virus) B-cell lymphoma 27 years later (unpublished data). This patient had a mild T-cell lymphopenia at that time. It is possible that microbial proliferation in the gastro-intestinal tract could favor the occurrence of lymphomas, as it does in other immunodeficiencies. It will be of crucial importance to register these cases to assess that risk.

***Table 1.* Hematopoietic stem cell transplantation for NK^-B^+ severe combined immunodeficiency**

T cells	<1000/μl	7/21
$CD4^+$ T cells	<300/μl	5/20
Naïve $CD4^+$ T cells	<100/μl	6/20
Natural killer cells	<50/μl	8/19
Clinical manifestations		10/21
Retransplanted (boost)		2

Immunological status, follow-up >10 years.

Improvement of immune functions can be provided by an HSCT boost. Infusion of mature T cells together with HSCT from an HLA-identical sibling rapidly restores immunity with clinical benefit (56, 59). This effect is much more unclear for those patients transplanted with the marrow from a haplo-identical donor, given the risk of thymus inefficacy (discussed above).

In order to avoid secondary loss of T-cell responses, newly diagnosed SCID patients should be treated in a way so that functional HSCs, those able to give rise to T lymphocytes (as well as NK and B lymphocytes), are present and persist. There are two options to be discussed: (i) allogeneic HSCT preceded by myeloablation in order to ensure donor stem cell engraftment and (ii) gene therapy, provided that early pluripotent progenitors can be efficiently transduced.

There are multiple examples of long-term survival of SCID patients who received an HSCT preceded by full myeloablation (high-dose busulfan and cyclophosphamide) and who have stable functional T-cell functions (22, 60–62). However, such an approach carries a very high risk of lethal toxicity in infected children. It might thus be selectively used in patients with mild or no infection and in NK^+ SCID patients who are at higher risk of graft failure.

A more satisfactory approach would be the utilization of a reduced-intensity conditioning (RIC) regimen, which presumably carries a lower risk of toxicity. This approach has been tried in Europe, by using a total dose of 8 mg/kg of busulfan and 200 mg/kg of cyclophosphamide. This regimen was well tolerated but did not significantly enhance donor stem cell engraftment in NK^+ or in B^+ SCID (25, 52, 54). This negative result does not exclude the possibility of finding another RIC regimen with better efficacy (63). The alternative is gene therapy, now made possible by the identification of almost all genes associated with SCID and the availability of retroviral vectors able to transduce hematopoietic progenitors.

Gene therapy of SCID

It has been reasoned that SCID would be a favorable condition for gene therapy, because the number of required transduced precursors is low, given the fact that gene transfer confers a tremendous growth-selective advantage to T-cell precursors and mature T cells are long lived (64). By using

Moloney-derived retroviral vectors, *in vitro* feasibility has been demonstrated for SCID-X1 (65–68) and *in vivo* for SCID-X1 (69–72), JAK-3 deficiency (73, 74), and Rag-2 deficiency (75). These experiments confirmed that retrovirus-mediated gene transfer of murine HSC corrected the SCID phenotype and that a selective advantage for lymphocyte precursors was observed.

Based on these findings, a clinical trial for SCID-X1 was initiated in 1999. The protocol has been described elsewhere (5) and is quite simple in its principle. Patients with no HLA-identical sibling were eligible, and no myeloablative treatment was given. Among the 10 treated patients with typical SCID-X1, T-cell development occurred in nine (5, 31, 49). A correlation between quality of T-cell reconstitution and number of transduced $CD34^+$ cells infused was detected (*Fig.* 6).

Suboptimal T-cell development occurred in two patients who received 1×10^6 $CD34^+\gamma c^+$/kg, whereas the T-cell pool fully developed in those who received $\geq 3 \times 10^6$ $CD34^+\gamma c^+$/kg, providing a minimal threshold of total number of cells to inject. These results have since been confirmed in four additional patients treated by Thrasher *et al*. (76) in London with a similar protocol. Characteristics of transduced T cells found in the patients are indicated in *Table* 2.

The T-cell repertoire is highly diversified, as $TREC^+$ T cells can still be detected 5 years after gene therapy in the first treated patients. Together with the observation that a few (1%) transduced myeloid cells can be detected over time, including $CD34^+$ cells (31, 49, and unpublished data), evidence for the persistence of transduced pluripotent progenitors cells is provided. This result has been confirmed by the findings of provirus integration sites shared by T and B lymphocytes, myeloid cells, and long-term cultured initiating cell-derived colonies (manuscript submitted). These results give hope for a long-term sustained T-cell immunity, which could potentially be of much longer duration than what is observed after HSCT. Of course, long-term monitoring will be required to assess whether this prediction turns out to be valid. Until now, correction of the immunodeficiency, which also includes in part B-cell immunity, is good enough so that patients normally cope with infections, including those caused by VZV, and live normally without therapy.

Efficacy of gene therapy also has now been reported in the treatment of four patients with ADA deficiency by using a similar methodology (77). Usage of a low-dose myeloablative therapy (busulfan, 4 mg/kg) might have increased the observed rate of myeloid cell transduction, thereby potentially improving long-term efficacy. These results open the door for an extension of gene therapy to other SCID conditions, as the growth selective advantage concept should apply, albeit with a variable intensity (78) to all forms of SCID.

There are some limitations to gene therapy of SCID. Failure of immunodeficiency correction occurred in a child with SCID-X1 who had an enlarged spleen caused by a disseminated Bacille Calmette-Guerin virus infection (31). As based on spleen analysis upon removal, transduced cells were likely trapped in the spleen, thus impairing T-cell differentiation

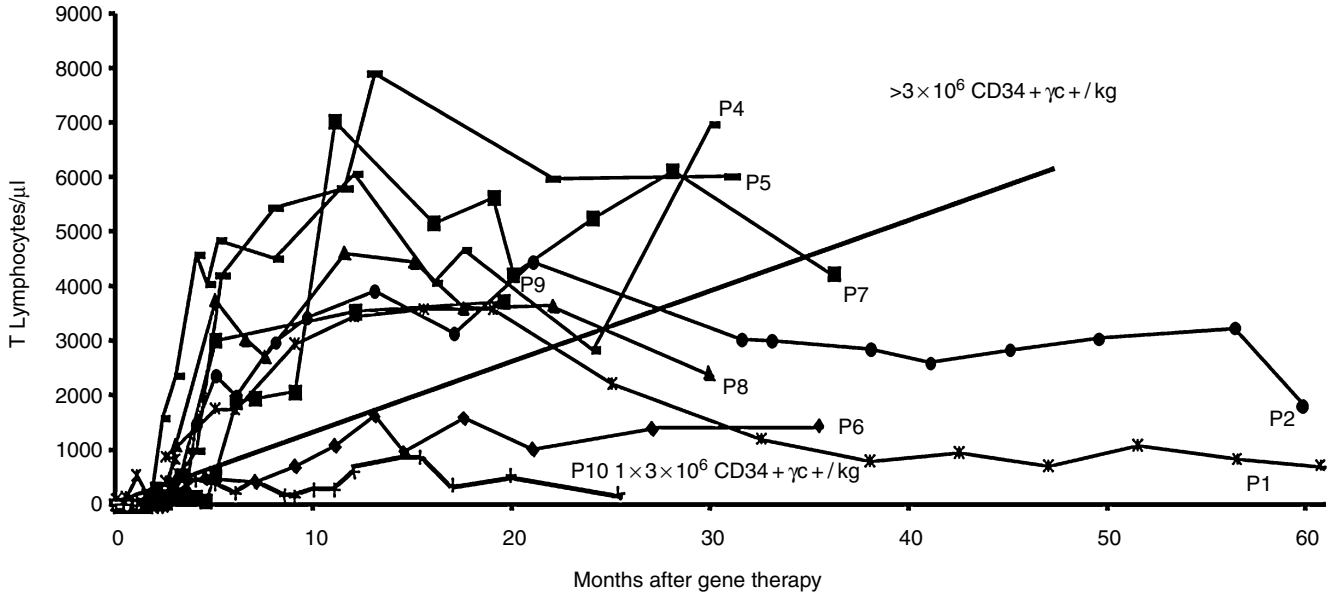

***Fig. 6.* T-lymphocyte development after gene therapy for severe combined immunodeficiency-X1.** P1→10: each curve represents T-cell counts for a given patient. In P6 and 10, who received 1×10^6 γ-chain $(\gamma c)^+CD34^+$ cells/kg, T-cell development was suboptimal (below the line) as compared to the other patients who received $>3 \times 10^6$ γc^+CD34^+ cells/kg.

***Table 2*. Severe combined immunodeficiency-X1 gene therapy – characteristics of T-cell pool reconstitution**

T-cell development	Yes
Kinetics	2.5–3 months
Function	Yes
Diversity	Broad repertoire
Persistence	<5.0 years
Nature of transduced cells	Multipotent progenitors
Transduced cells→T cells	Approximately 10^2

into the thymus. Therefore, such (rare) patients might require splenectomy prior to be eligible for gene therapy.

Gene therapy in older SCID-X1 patients, either patients with an incomplete phenotype or a patient in whom HSCT at least partially failed, did not succeed (79). This failure is likely the consequence of the premature loss of the thymic function in SCID patients in the absence of functional T-cell precursor cells. There is probably only a relatively narrow window of a few years, which remain to be more precisely determined, during which gene therapy as a primary (or secondary) strategy can be used. Of note, this restriction also applies to haploidentical HSCT.

Two patients from our SCID-X1 gene therapy trial developed clonal T-cell proliferation, as described in detail elsewhere (49). The primary cause was insertion of the provirus within the LMO-2 locus, leading to aberrant expression of LMO-2 in mature T cells and thereby uncontrolled proliferation (80). It was found that this serious adverse effect carries a higher risk to occur in the context of SCID-X1, as also suggested in a murine model of leukemogenesis (81), and hence this therapy should be restricted to patients of the youngest age (82). Characteristics of hematopoiesis at an early age may favor provirus integration in such sites. This is why today it is considered that the benefit/risk balance of gene therapy in SCID-X1 as compared to haploidentical HSCT can be regarded as positive at least for patients above a certain age (4–6 months). There are SCID-X1 gene therapy trials open in Paris, London, and the US National Institutes of Health. Further application of gene therapy in SCID nevertheless should try to use potentially safer vectors, including self-inactivated long terminal repeats and perhaps insulators and a rescue 'suicide' gene (83).

Conclusion

SCID has been at the forefront of experimental medicine over the last 35 years or so because of its intrinsic high risk of lethality and the growing progress in understanding its molecular bases. Advances in diagnosis and therapy have been significant. It is quite likely that, for the benefit of the patients, new therapeutics will be developed for SCID, paving the way for application in other fields of medicine.

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