Defining combined immunodeficiency

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Background: Although the extreme condition of typical profound T-cell dysfunction (TD), severe combined immunodeficiency (SCID), has been carefully defined, we are currently in the process of better defining less typical T-cell deficiencies, which tend to present with autologous circulating T-cell combined immunodeficiency (CID). Because autologous cells might interfere with the outcome of bone marrow transplantation, protocols usually include conditioning regimens. Therefore it is important to define the numbers of autologous cells usually detected in patients with CID versus those with SCID.

Objectives: We sought to determine the number of circulating T cells in patients with SCID as opposed to those with CID, to study their function, and to evaluate their possible detection during newborn screening using T-cell receptor excision circle (TREC) analysis.

Methods: Numbers of circulating $CD3^+$ T cells (as determined by means of flow cytometry), *in vitro* responses to PHA, and TREC levels, all measured at presentation, were compiled from the research charts of the entire cohort of patients followed prospectively for T-cell immunodeficiency at the Hospital for Sick Children. Clinical data were ascertained retrospectively from the patient's hospital charts.

Results: One hundred three patients had $CD3^+$ determinations, and 80 of them had a genetic diagnosis. All patients considered to have typical SCID had $CD3^+$ T-cell counts of fewer than 500 cells/μL. Some variability was observed among different genotypes. *In vitro* responses to PHA were recorded in 88 patients, of whom 68 had a genetic diagnosis. All patients with low $CD3^+$ T-cell numbers (<500 cells/μL) also had markedly decreased responses to PHA (typical SCIDs). However, responses ranged widely in the groups of patients with TD who had more than 500 $CD3^+$ autologous circulating T cells per microliter. Although patients with Omenn syndrome and ζ chain–associated protein, 70 kDa *(ZAP70)*, and purine nucleoside phosphorylase (PNP) deficiencies had low responses, patients with the p.R222C mutation in the IL-2 receptor γ *(IL2RG)* gene as well as IL-10 receptor and CD40 ligand deficiencies had normal or near-normal mitogen responses. Finally, 51 patients had TREC levels measured. All patients with typical SCID, Omenn syndrome, and *ZAP70* deficiency had low TREC levels. In contrast, patients with mutations in forkhead box protein 3 *(FOXP3)*, CD40 ligand *(CD40L)*, and IL-10 receptor α *(IL10RA)*, as well as patients with the p.R222C mutation in the *IL2RG* gene, had normal TREC levels.

Conclusion: Patients with typical SCID can be defined as having fewer than 500 circulating $CD3^+$ T cells. Most patients with autologous T cells still have profound TD, as defined by reduced *in vitro* function and thymus output. Some patients with conditions including TD have normal TREC levels and will therefore not be detected in a TREC-based newborn screening program. (J Allergy Clin Immunol 2012;130:177-83.)

Key words: *Combined immunodeficiency, CD3, phytohemagglutinin, T-cell receptor excision circles*

Severe combined immunodeficiency (SCID), an extreme form of T-cell deficiency with or without B-cell deficiency and sometimes also low natural killer cell numbers, typically presents in infancy with pneumonitis, chronic diarrhea, and failure to thrive. Physical examination reveals a lack of palpable lymph nodes, oral thrush, and chest imaging that is remarkable for a lack of thymus shadow in most cases. The laboratory hallmark in these cases is profound T-cell lymphocytopenia. However, the number of circulating T cells can vary among patients, and there is no clear definition of what constitutes T-cell lymphopenia in patients with SCID.

In the past 2 decades, we have witnessed the discovery and characterization of a growing number of patients who present as patients with SCID with repeated opportunistic infections, but unlike patients with SCID, they have a significant number of host origin (autologous cells) circulating T cells.[1-7] Frequently, these patients have a prominent skin rash, enlarged lymph nodes, and a normally sized thymus.[7,8] Some of these patients can have a delayed or atypical presentation beyond 1 year of age or have lung granulomas[9] or lymphoid malignancy,[10] whereas others might have associated syndromic features. The terms T^+ SCID, combined immunodeficiency (CID), or leaky SCID have been frequently used to describe this group of inherited immune disorders, which all share profound T-cell dysfunction (TD).

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Abbreviations used
ADA: Adenosine deaminase
CD40L: CD40 ligand
CHH: Cartilage-hair hypoplasia
CID: Combined immunodeficiency
FOXP3: Forkhead box protein 3
HSCT: Hematopoietic stem cell transplantation
IL2RA: IL-2 receptor α
IL2RG: IL-2 receptor γ
IL7RA: IL-7 receptor α
IL10RA: IL-10 receptor α
JAK3: Janus kinase 3
PJP: *Pneumocystis jiroveci* pneumonia
PNP: Purine nucleoside phosphorylase
RAG: Recombination-activating gene
RMRP: RNA component of mitochondrial RNA processing endoribonuclease
SCID: Severe combined immunodeficiency
SI: Stimulation index
TD: T-cell dysfunction
TREC: T-cell receptor excision circle
ZAP70: ζ Chain–associated protein, 70 kDa

This heterogeneous group of disorders includes (1) patients who carry genetic aberrations that typically present with autologous T cells, (2) leaky SCID caused by hypomorphic mutations in SCID-causing genes, (3) multiorgan syndromes associated with TD, and (4) TD with a yet unknown genetic defect.

The first group includes TD disorders in which all known mutations are consistently associated with circulating autologous T cells. Patients with mutations in ζ chain–associated protein, 70 kDa *(ZAP70)*[1]; *CD3G*[11]; and IL-2 receptor α *(IL2RA)*[2,12] are good representatives of this group.[13] Despite having a large number of circulating T cells, *in vitro* responses to anti-CD3 antibody or mitogens are frequently decreased. Some features of these patients might aid in the diagnosis. CD8 lymphocytopenia might suggest a mutation in *ZAP70*,[14] whereas the lack of an *in vitro* response to exogenous IL-2 or reduced expression of $CD3^{-}$ might point to the possibility of mutated *IL2RA* or *CD3G*, respectively. Severe intractable gastrointestinal manifestations presenting early in infancy[13] could be signs of deficiencies in the *IL2RA*,[2] forkhead box protein 3 (*FOXP3*),[15] or *IL10R*[16] genes.

The second group of patients consists of those with TD caused by mutations (hypomorphic) that allow for a substantial "leak" of T cell from the thymus. Some of these patients present with severe erythroderma, eosinophilia, lymphadenopathy, and hepatosplenomegaly, the hallmarks of Omenn syndrome. Invariably, such patients have a restricted T-cell repertoire with oligoclonal T-cell expansion. A molecular diagnosis can be made in the majority of these patient, including hypomorphic mutations in recombination-activating gene *(RAG)* 1/2,[17] *DCLRE1C*, *LIG4*,[17-19] RNA component of mitochondrial RNA processing endoribonuclease *(RMRP)*,[7] IL-2 receptor γ *(IL2RG)*,[20] IL-7 receptor α *(IL7RA)*,[21] or adenosine deaminase *(ADA)*.[22]

More difficult to diagnose are cases with hypomorphic mutations in SCID-causing genes but with no features of Omenn syndrome. Most frequently, these patients also have a full T-cell repertoire or near-normal representation of all Vβ families. Typical examples of these cases are patients who carry the p.R222C mutation in the *IL2RG* gene. Some of these patients might have normal lymphocyte counts and near-normal *in vitro* mitogenic responses.[4,5]

Profound immunodeficiency can also be associated with various multisystem syndromes, such as ADA deficiency,[22] cartilage-hair hypoplasia (CHH),[7] or DNA ligase 4 deficiency.[23]

Finally, there are still many cases of TD in which the genetic basis of the condition remains unknown. Importantly, some patients with SCID might have variable levels of circulating T cells of maternal origin. Such patients can have a graft-versus-host disease–like skin rash similar to Omenn syndrome. Therefore it is important to examine this possibility in all patients with TD who have circulating T cells.

The diagnosis of CID can be challenging because of the presence of autologous circulating T cells with various degrees of residual function. To date, we have no clear definitions for this group of patients, which makes it difficult to formulate a uniform approach to treatment, such as the use of conditioning before hematopoietic stem cell transplantation (HSCT). We have attempted to define better this group of patients by comparing them with patients with typical cases of SCID.

We show here that cases of CID and SCID can be differentiated from each other not only on the basis of CD3 counts but also based on the type of clinical presentation and the ability to be detected by using newborn screening with T-cell receptor excision circles (TRECs).

METHODS

The definition of profound T-cell deficiency included all conditions with evidence of potentially fatal primary T-cell deficiency severe enough to require replacement of the immune system with HSCT (or other alternatives [ie, gene therapy]). The diagnosis in most patients was based on severe lymphopenia, decreased *in vitro* responses to mitogens, or both. Other patients were given diagnoses according to a combination of typical clinical features, such as erythroderma, T-cell clonal expansion in patients with Omenn syndrome, extreme short stature in patients with CHH, or microcephaly for those with DNA ligase 4 deficiency. In rare cases thymus biopsy specimens (mutated *ZAP70* and *IL2RA*) or skin grafts (p.R222C mutant of *IL2RG*) were used. Molecular analysis has aided in the diagnosis in recent years.

Data were compiled from prospective and ongoing research charts, as well as the database of the Canadian Centre for Primary Immunodeficiency. This includes consent and assent from patients and parents for genetic analysis, collection of tissue, and establishment of immortalized EBV-infected B-cell lines and fibroblast cell lines. Molecular analysis both at the time of diagnosis and after diagnosis and treatment was included in a prospective study (protocol no. 1000005598). Clinical data were also collected retrospectively from medical records for all patients to ascertain the accuracy and completeness of the data (protocol no. 1000010972). One hundred three patients with TD were included in this study with the approval of the institutional research ethics review board. This cohort included all patients who were given a diagnosis in the first 3 years of life of TD at the Hospital for Sick Children between 1988 to 2011. All had records of CD3 staining determined by means of flow cytometry, as previously described.[1,2,4,6] Eighty-eight of the 103 patients also had *in vitro* mitogenic responses to PHA, as previously described,[1,2,4,6] and 51 of the 103 patients had TREC levels determined, as previously described.[24] Forty-three patients had all 3 values available. The range of $CD3^{+}$ T-cell numbers that best distinguish between SCID and CID was determined by analyzing the sensitivity and specificity at various $CD3^{+}$ T-cell thresholds. Statistics were evaluated by using the Fisher exact test and considered significant if the double-sided *P* value was less than .05.