CD3 deficiencies

Alain Fischer[a], Geneviève de Saint Basile[a] and Françoise Le Deist[a,b]

Purpose of review

The molecular characterization of inherited T-cell immunodeficiencies has contributed to delineating key factors in human T-cell development. This review reports on the recent description of deleterious mutations in the genes encoding CD3 subunits expressed at the T-lymphocyte membrane in association with the T-cell receptor.

Recent findings

Homozygous mutations in CD3D and CD3E genes lead to a complete block in T-cell development and thus to an early-onset severe combined immunodeficiency phenotype. Thymic studies have shown that the defect in T-cell development occurs at the transition between 'double-negative' and 'double-positive' thymocytes. These results contrast with the partial T-cell immunodeficiency caused by a deficiency in CD3G.

Summary

Two new severe combined immunodeficiency conditions have been reported as a consequence of either CD3D or CD3E deficiency. The distinct phenotype of CD3G deficiency sheds light on the differential roles of CD3 subunits in T-lymphocyte development.

Keywords

severe combined immunodeficiency, T-cell receptor/CD3 subunits/T-cell immunodeficiency

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[a]Department of Pediatric Immuno-Hematology and Inserm Unit 429, Necker–Enfants Malades Hospital, Paris, France and [b]Department of Microbiology and Immunology, Sainte Justine Hospital, Montréal, Canada

Correspondence to Alain Fischer, Necker–Enfants Malades Hospital, 149 Rue de Sèvres, 75015 Paris, France
Tel: +33 1 44 49 50 71/1 44 49 48 22; fax: +33 1 42 73 06 40;
e-mail: alain.fischer@nck.ap-hop-paris.fr

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Abbreviations

ITAM	immunoreceptor tyrosine-based activation motif
SCID	severe combined immunodeficiency
TCR	T-cell receptor

1528-4050

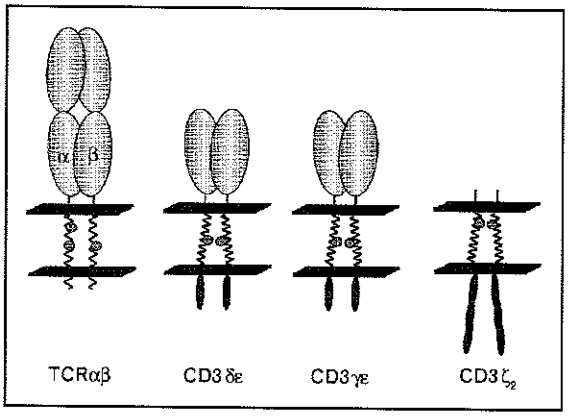
Introduction

The study of genetic defects resulting in an absence of mature T cells in humans has provided major insights into our current understanding of T-lymphocyte development. These conditions are named 'severe combined immunodeficiencies' (SCIDs) because they result in an impairment of both T and B-cell-mediated immunity. Four major mechanisms causing SCID have been reported [1], including (1) premature lymphoid progenitor cell death because of abnormal purine metabolism (adenosine deaminase deficiency); (2) a block in lymphokine signaling (γc, JAK-3 and IL7-Rα deficiencies); (3) defective V(D)J rearrangement of T-cell receptor (TCR) and B-cell receptor genes (Rag-1, Rag-2, Artemis); and (4) defective pre-TCR/TCR signaling. In this last subset, deficiencies in the CD45 tyrosine phosphatase as well as in CD3 subunits of the TCR/CD3 complex have been described [2,3•]. This review is focusing on CD3 deficiencies. They account for a small proportion of SCID conditions. Despite their rarity, the analysis of their phenotypes reveals the complexity of the functions associated with the TCR/CD3 complex.

The TCR/CD3 complex

The antigen recognition unit of T cells is based on a heterodimer composed either of the α/β chain of the TCR or of the γ/δ chain [4•] (Fig. 1). The TCR heterodimer is associated with four transmembrane proteins, respectively named CD3 δ, γ, ε and ζ [5]. They form a complex with the following stochiometry CD3 δ, γ, ε_2, ζ_2 [6•]. To date, according to both functional and structural data, it is thought that the CD3 complex is formed of three modules CD3 δ/ε, CD3 γ/ε and CD3 ζ_2 [7]. The $\gamma\delta$ CD3 subunits have acidic residues in their transmembrane domains enabling them to interact with the positive charges of the TCR subunits to form a complex. All of the CD3 subunits carry the so-called immunoreceptor tyrosine-based activation motif (ITAM) in the intracytoplasmic region. CD3 δ, γ and ε have one ITAM, whereas CD3 ζ has three. These ITAMs are composed of two tyrosines separated by nine to 12 residues. Upon ligand binding to TCR, ITAMs get phosphorylated on tyrosine by the src-related kinase lck. Phosphorylated ITAMs can recruit the zeta-associated kinase (ZAP-70), which initiates key downstream events in TCR-mediated signaling, including the recruitment of SLP-76 and LAT adaptor molecules [8]. The CD3 subunits are also associated with the pre-TCR expressed by thymocytes, which is composed of the TCRβ and the surrogate ptα chain [9]. Pre-TCR

Figure 1. Schematic structure of the T-cell receptor ($\alpha\beta$ or $\gamma\delta$) associated with the CD3 subunits.



CD3 subunits form three distinct 'functional' nodules CD3 δ/ε, CD3 γ/e and CD3 ζ [2]. TCR, T-cell receptor.

signaling is required towards progression to the CD4+ CD8+ stage and TCRα gene rearrangement [9].

A deficiency of either one of the CD3 δ, γ and ε has been found in patients with primary immunodeficiency diseases. Although very rare, these cases provide unexpected and useful information on the role of the respective CD3 subunits.

CD3 δ deficiency

In six patients from three unrelated families, a CD3 δ deficiency has been found as the cause of a T(−) NK(+) B(+) phenotype of SCID [2,3•]. Two affected fetuses in one family were also studied after pregnancy termination [3•]. In all cases, the absence of mature T cells led to an early occurrence (first months of life) of severe infections. The phenotypic consequences of CD3 δ deficiency are clear-cut because no mature CD3(+) T cells can be detected in the periphery, including both TCR $\alpha\beta$(+) and $\gamma\delta$(+) T cells. An examination of fetal tissues confirmed the absence of T cells in the lymph nodes in contrast to the normal detection and distribution of B cells [3•].

In two families, the identification of *CD3D* gene mutations was based on gene mapping by linkage analysis showing co-segregation of the disease with markers at the 11q23 locus encompassing *CD3 D*, *E* and *G* genes. In the third family, micro-array analysis of gene expression in the thymus led to the discovery that *CD3D* gene expression was low. The same homozygous C202T transition in exon 2 has been found in two unrelated families from Mennonite and north African origin, respectively. It led to a C68X stop codon generation expected to truncate the CD3 δ protein in its extracellular domain, thus impairing membrane association and expression [2,3•]. Heterozygotes had a normal TCR/CD3 complex expression on T cells and are healthy, showing the autosomal recessive inheritance of this condition. In the third family, a homozygous C279A transversion in exon 3 was found in two siblings. It also leads to a stop codon (C93X) located in the extracellular domain of CD3 δ. In all six patients (and two affected fetuses), a homozygous *CD3D* gene mutation thus led to a null phenotype.

Thymic biopsies from an affected infant and two affected fetuses (at 22 weeks of gestation) were analysed for the T-cell differentiation pathway. In the former case, the expression of messenger RNA and proteins associated with T-cell development was analysed, whereas in the latter immunohistology was performed. In the CD3 δ-deficient patient, CD4 and CD8 $\alpha\beta$ mRNA and proteins as well as TCRα and β mRNA and proteins were low, indicating a block at the transition from double-negative to double-positive (CD4+ CD8+) thymocytes, whereas CD3 subunits mRNA were detectable. In the fetuses, thymocytes with an intermediate single-cell positive phenotype, i.e CD4(+) CD8(−) CD45 RO(−) were detected. Cellularity was reduced and the fraction of proliferating cells (Ki 67+) was very low. Together, these data fit with a model placing the CD3 δ deficiency-induced block at the stage of pre-TCR signaling, although, at least in the 22-week-old fetuses, cells can differentiate a little further to the intermediate single-cell positive stage (but cannot proliferate) (Fig. 2) [10].

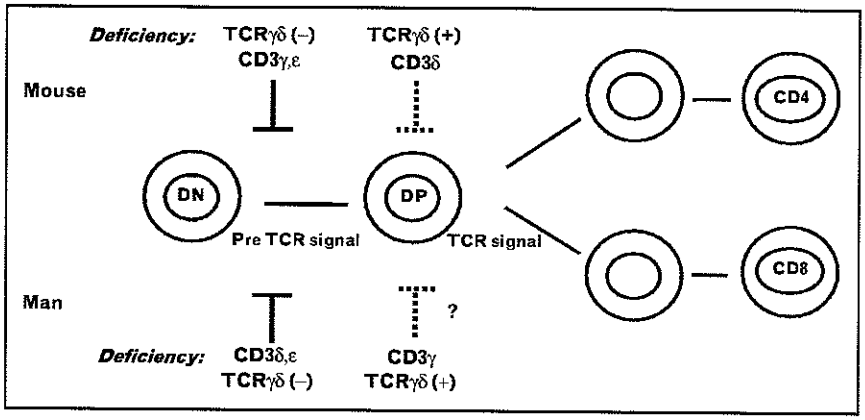
It is interesting to compare this phenotype with the one of CD3 $\delta^{-/-}$ mice. These mice exhibit a partial block at a later stage of thymocyte differentiation, namely at the double-positive T-cell stage, whereas some mature T cells with a normal phenotype can be detected in the periphery [11]. Moreover, CD3 $\delta^{-/-}$ mice do have TCR γ/δ+ T cells unlike CD3 δ-deficient patients (Fig. 2) [11,12]. These results clearly establish that murine and human CD3 δ exert distinct functions in T-cell development, despite a highly conserved structure and sequence between both species. In humans, the CD3 δ/ε heterodimer is required for early steps of TCR γ/δ development as well as for pre-TCR-mediated progression towards TCRα rearrangement and the CD4+/CD8+ double-positive stage, whereas it appears to be dispensable in the murine counterpart. These observations also demonstrate that the functional modules are not redundant and therefore have specific functions that remain to be identified. So far, only a CD3 δ-dependent activation of the erk kinase in the positive selection of double-positive thymocytes has been shown in mice [13].

CD3 ε deficiency

A complete CD3 ε deficiency has recently been found in two patients from a consanguineous family, whereas

Figure 2. Developmental blocks in T-cell differentiation caused by CD3 subunit deficiencies in humans and mice.

Scheme of T-cell development is simplified in three steps: double-negative (DN) CD4− CD8− thymocytes that proliferate in response to γc-containing cytokine receptors, progression to double-positive (DP) T-cell receptor (TCR)α β T cell (CD4+ CD8+) through signaling via the ptα TCRβ receptor, then positive selection and generation of single-positive (SP) CD4+ and CD8+ αβ+ T cells [10].



partial CD3 ε deficiency has been observed in an unrelated third patient with a milder immunodeficiency [3•,14]. CD3 ε deficiency was found to result in a complete absence of mature TCR αβ and γδ T cells in the periphery whereas natural killer and B lymphocytes were normally detected. The clinical consequences, as in other forms of SCID, do appear in the first months of life (respectively 1.5 and 2 months) with severe infections: cytomegalovirus pneumonitis, diarrhoea and candidiasis. In the family with complete CD3 ε deficiency, genome linkage analysis indicated that the disease locus was at 11q23, leading to the identification of a homozygous 2 base pair deletion at position 128 in exon 5 of the *CD3E* gene, resulting in a frameshift and the generation of a premature stop codon [3•]. The predicted protein product, which lacks the transmembrane domain, is not expected to be expressed at the cell membrane. As no material was available, the precise consequences of CD3 ε deficiency in human thymocyte development could not be assessed. In CD3 $\varepsilon^{-/-}$ mice, there is an absolute block at the CD44− CD25+ double-negative (DN3) stage, indicating an absence of a pre-TCR-mediated signal (Fig. 2) [15,16]. It is likely that there is an identical block in T-cell development in humans.

In our experience, CD3 δ and CD3 ε deficiencies, both of which result in a T(−), B(+) NK(+) SCID phenotype, account for five out of 160 T(−) SCID cases, i.e. 3% [1]. As there are a few other T(−) B(+) NK(+) SCID cases with as yet no associated identified molecular defects, it may be postulated that a deficiency of other members of the TCR/CD3 complex (or other molecules crucial for T-cell development) can also result in this SCID condition.

A partial CD3 ε deficiency has been identified in a 2-year-old patient presenting with recurrent bronchopneumonitis caused by *Haemophilus influenzae* [17]. Under intravenous immunoglobulin substitution, he did not develop other infections, notably no opportunistic infections. He is presently 18 years old, doing well other than suffering with severe sinusitis. Immunological work-up of this case revealed a low intensity expression (reduced by one log) of the TCR/CD3 complex expression at the T-cell surface. T cells were otherwise phenotypically normal as were the other lymphoid lineages [18]. T cells could be activated *in vitro* to proliferate in response to antigens, although CD3-mediated activation was impaired [18]. Biochemical and genetic analysis led to the identification of a CD3 ε deficiency caused by compound heterozygous mutations of the CD3E gene inherited from unrelated parents. The first consists of a null allele caused by a G → A transition in exon 6, leading to a stop codon in the extracellular domain, whereas the second consists of a splice site mutation in the donor site of intron 7 (second nucleotide t → c), which partly impaired splicing and resulted in a mRNA lacking exon 8 [14]. A partly preserved normal splicing permitted the generation of approximately 10% of the normal amount of the CD3 ε subunit. It is interesting to see that the functional consequences of this CD3/TCR expression deficiency were limited (see above). This suggests that T cells with a high affinity for peptide/major histocompatibility complex could probably be selected despite a reduced expression of the TCR, indicating that a normal density of TCR expression is not strictly required. Defective T-cell-mediated help for antibody production was the most remarkable immune defect in this case.

It is not unlikely that other partial defects in TCR/CD3 expression/function do exist. An abnormal pattern of expression should lead to a search for them. One cannot, however, exclude the possibility that more subtle abnormalities that do not impair TCR/CD3 complex

assembly and expression, but perhaps signaling only (e.g. mutation in the intracellular domain notably ITAM elements) can also result in mild T/B immunodeficiencies.

CD3 γ deficiency

A CD3 γ deficiency has been found in three patients from two families, one from Spain [18], and one from Holland in a patient of Turkish origin [19]. In the first family, a sibling died at 32 months of age from autoimmune haemolytic anaemia and enteropathy, whereas the second has been reported to be almost healthy at the age of 19 years in 2000 [20]. He only has minor autoimmune-related disease: vitiligo and the detection of auto anti-thyroid antibodies. The third case from an unrelated family was reported to be healthy at the age of 7 years. Anti-thyroid antibodies were also detected in this patient.

This mild immunodeficiency is associated with a reduced expression of the TCR/CD3 complex at the T-cell surface (1 : 4 to 1 : 5 of normal) [18]. Notably, CD8 T-cell counts were reduced unlike CD4 and other T-cell subsets. A mild reduction in in-vitro T-cell activation assays was noticed. Of note is the fact that most T cells exhibit a memory phenotype suggesting that thymopoiesis might have been only transiently active [19–21].

In contrast to the limited expression of this immunodeficiency phenotype, compound heterozygous mutations inherited from the parent resulted in a null CD3γ phenotype in both patients from the first family. One allele consists of an A → G transition in the first codon of exon 1 impairing the initiation of translation. The second mutation is a g → c mutation in the acceptor splice site of intron 2 leading to its loss, and the usage of a cryptic splice site 17 nucleotides downstream ensues. A shift in the reading frame, and the creation of a stop codon 5 nucleotides downstream ensues. Mutations in the CD3G gene of the third patient are not published.

Further analysis of the immortalized CD3 $\gamma(-)$ T cells by using the herpes virus Saimiri reveals that a TCR/CD3 ε_2 δ_2 ζ_2 complex was formed and expressed, showing that in humans CD3 δ can partly substitute for CD3 γ. Ligand-induced endocytosis of such as a complex was found to be impaired, leading to delayed turnover and re-expression of the TCR at the cell surface, showing a role of CD3 γ in TCR/CD3 complex trafficking [22–25]. Such T-cell cells could be activated through TCR/CD3 agonists to flux calcium, express CD69, CD40 L, proliferate and produce TNF-α. However, IL-2 production was significantly reduced, a defect that could be overcome by protein kinase C activation through phorbol esters [25].

This mild phenotype contrasts strikingly with the one observed in CD3 γ-deficient mice. Such mice exhibit a complete block at the DN3 stage, like CD3 $\varepsilon^{-/-}$ mice, and do not have TCR $\gamma\delta$ T cells [26]. Surprisingly enough, there is thus an almost mirrored image between mice and humans (Fig. 2) for CD3 δ and γ function in T-cell development. It is now a challenge to understand how these highly conserved molecules exert distinct functions in mice and humans, and what accounts for the dissimilarities between the activation signals delivered by the highly similar CD3 δ/ε and CD3 γ/ε heterodimers, although there are indeed some recognizable structural distinctions [10].

Conclusion

The discovery of CD3 subunit deficiencies has further broadened the spectrum of molecular defects that may lead to a SCID phenotype in humans. This reinforces the need for more accurate molecular diagnosis and genetic counseling of these life-threatening conditions. Unexpected observations, as summarized in Fig. 2 set the basis to 'revisit' the function of the CD3 molecules in T-cell development and activation.

It is also very likely that by pursuing investigations of so far ill-defined SCID conditions and partial T+B immunodeficiencies, more will be learned about the TCR/CD3 complex.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 570–571).

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