

Zap-70 (zeta-chain-associated protein 70 kD) deficiency

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Abstract

Zap-70 (zeta-chain-associated protein 70 kD) deficiency is an autosomal recessive form of severe combined immune deficiency (SCID) that is characterized by lack of CD8+ T cells and normal presence of circulating CD4+ T cells. The disease is extremely rare, with hardly 12 patients from 8 unrelated families reported so far. Nearly all patients with Zap-70 defects presented with typical clinical features of SCID in early life: severe pulmonary infection often sustained by opportunistic pathogens (Pneumocystis carinii), chronic diarrhoea, failure to thrive, and persistent candidiasis. Zap-70 deficiency is ultimately fatal unless patients undergo bone marrow transplantation (BMT). In the future, gene therapy could appear to be an alternative form of treatment. Most of the ZAP-70 gene defects identified in humans prevent protein expression and are concentrated in a region that is critical for stability and enzymatic activity. Mutations include insertions, deletions, and substitutions of a single nucleotide. Antenatal diagnosis by analysis of chorionic villi DNA can be carried out.

Keywords

Severe combined immune deficiency, Zap-70, tyrosine kinase, lymphocytes.

Disease name - Synonyms

Zap-70 (zeta-chain-associated protein 70 kD) deficiency is a rare autosomal recessive form of severe combined immune deficiency (SCID) that is characterized by lack of CD8+ T cells with normal number of circulating CD4+ T cells that however are unresponsive to T-cell-receptor (TCR)-mediated stimulation *in vitro*. Although

immunological diagnosis shows CD8 deficiency, it cannot be called "CD8 deficiency" anymore, since a variety of gene defects leading to reduced number of CD8+ T lymphocytes have now been identified.

Excluded diseases

The term "Zap-70 deficiency" should be applied only to those cases with documented

biochemical and molecular abnormality of the Zap-70 protein or gene. All other forms of SCID (Buckley, 2004), that often cannot be distinguished clinically, should therefore be excluded.

Diagnostic criteria - Definition

The diagnosis of Zap-70 deficiency is prompted by recognition of typical clinical features of SCID associated with marked reduction of circulating CD8+ T cells and strongly reduced *in vitro* proliferative response to mitogens. However, a definitive diagnosis requires demonstration of lack of Zap-70 protein in lymphocytes by Western blotting and/or identification of ZAP-70 gene defects.

Differential diagnosis

Differential diagnosis includes all the other forms of SCID in humans. More specifically, differential diagnosis should be established versus those forms of SCID with reduced number of CD8+ T cells, such as transporter of antigen peptides TAP deficiency. In the latter, *in vitro* proliferative response to mitogens is normal and reduced (or even absent) expression of major histocompatibility complex MHC class I molecules is detected at cell surface.

Prevalence

The disease is extremely rare, with 12 patients from 8 unrelated families reported so far. Many of them, including the first case reported, (Roifman et al, 1989) were Indian Mennonites.

Clinical description

With one single exception (Katamura et al, 1999), all the patients with Zap-70 defects presented with typical clinical features of SCID in early life: severe pulmonary infection, often sustained by opportunistic pathogens (*Pneumocystis carinii*), chronic diarrhea, failure to thrive, and persistent candidiasis.

By contrast, the unique patient reported by Katamura *et al.* presented with infiltrative skin lesions, that were slightly exudative since birth. These skin manifestations were due to accumulation of activated, memory, and presumably self-reactive CD4+ T cells. This patient was identified as a compound heterozygote for two mutations that resulted in production of temperature-sensitive mutants that are rapidly degraded in the cytoplasm at 37°C, but might be expressed and induce activation of self-reactive T cells in the skin, where local body temperature is lower (Matsuda et al, 1999).

The immunological and molecular data of the patients reported so far have been collected in a specific database (ZAP70base), that is fully accessible at the following address: <http://www.uta.fi/imt/bioinfo/ZAP70base/>.

Management including treatment

Zap-70 is a form of SCID and is ultimately fatal, unless it is treated by bone marrow transplantation (BMT). Four patients have been reported to have undergone BMT: two siblings have been treated with bone marrow from matched unrelated donors (Monafo et al, 1992), one has undergone BMT from an HLA-matched family donor (Mazer, 1997), and the last infant has been treated with two consecutive haploidentical transplants (Elder et al, 2001). Three of these patients have been cured. Isolation in a protected environment and regular use of antibiotic prophylaxis (trimethoprim(TMP)-sulfamethoxazole(SMZ): 5 mg/Kg TMP, 25 mg/Kg SMZ three alternate days a week) and of intravenous immunoglobulins (400 mg/Kg/21 days) are required while planning for BMT.

Recently, Fagioli F *et al* reported successful unrelated cord blood transplantation in child with severe Zap 70 deficiency; this therapy represents a valid alternative source of haemopoietic stem cells. (Fagioli F *et al.*, 2003) Retroviral-mediated insertion of the ZAP-70 gene into Zap-70-deficient CD4+ cells *in vitro* has resulted in functional reconstitution, with a selective growth advantage of transduced cells (Taylor et al, 1996; Steinberg et al, 2000), indicating that potential gene therapy could be an alternative form of treatment in this disease.

Since 2003, although gene therapy was highly successful in nine infants with X-linked SCID, the trials have been placed on hold due to the development of a leukemic process in two of the children because of insertional oncogenesis.

(Hacein-Bey-Abina S *et al*, 2003, Chinen J *et al.*, 2004)

Etiology

Zap-70 is an intracellular tyrosine kinase that is recruited in the CD3 T-cell receptor (TCR) complex and is required for T-cell activation following TCR engagement. TCR-mediated signaling induces Zap-70 recruitment of Zap-70 to the ζ chain of the TCR-CD3 complex, followed by Zap-70 phosphorylation and activation. Activated Zap-70 can then phosphorylate linker of activated T cells (LAT) and the Src homology 2 domain-containing 76-kD a leukocyte protein (SLP-76), allowing recruitment and activation of other critical signaling molecules, such as phospholipase C γ 1 (PLC γ 1) and Vav (guanine nucleotide exchange factor) (van Leeuwen *et al.*, 1999). These reactions are required for mobilization of intracellular free calcium [Ca $^{2+}$]_i and activation of the phosphatidylinositol-3-kinase and the Ras/MAP kinase pathways, and ultimately culminate in T-cell activation and

initiation of T-cell-specific responses (Qian *et al.*, 1997; van Leeuwen *et al.*, 1999).

Defective expression and/or function of Zap-70 results in impaired T-cell activation and affects T-cell development and function. Zap-70 is expressed since early stages of thymocyte development, and Zap-70 deficiency profoundly affects T-cell development. The observed generation of CD4+ T cells in humans (but not in mice) with Zap-70 deficiency is justified by a different requirement for integrity of Zap-70-mediated signaling in CD8+ versus CD4+ thymocytes (Gelfand *et al.*, 1995). However, once mature CD4+ T cells have been generated, they are fully dependent on Zap-70 for cell activation; hence, the severe defect of T-cell proliferative responses observed in infants with Zap-70 deficiency. This fact is observed in patients with Zap-70 deficiency but not in mice.

Most of the *ZAP-70* gene defects identified in humans prevent protein expression and are concentrated in a region that is critical for stability and enzymatic activity. Mutations include insertions, deletions, and single nucleotide substitutions (Chan *et al.*, 1994; Arpaia *et al.*, 1994; Elder *et al.*, 1994; Noraz *et al.*, 2000; Matsuda *et al.*, 1999). Recently, the first example of a *Zap-70* defect (R465C) that selectively affects kinase activity but does not prevent protein expression, has been reported (Elder *et al.*, 2000).

Diagnostic methods

The main laboratory features of Zap-70 defect include selective absence of circulating CD8+ T cells associated with markedly reduced or absent *in vitro* proliferative responses of peripheral blood lymphocytes to phytohemagglutinin, anti-CD3 monoclonal antibodies or alloantigens. By contrast, stimulants such as phorbol esters and ionomycin, that bypass membrane-proximal cell signaling, result in normal lymphocyte proliferative responses. Serum levels of immunoglobulins may be normal (Roifman *et al.*, 1989; Monafa *et al.*, 1992; Katamura *et al.*, 1999) or low (Gelfand *et al.*, 1995; Elder *et al.*, 1996; Elder *et al.*, 2001). Variable results have been also reported for antigen-specific antibody production (Roifman *et al.*, 1989; Mazer *et al.*, 1997). Thymic biopsy reveals normal presence of double positive (CD4+ CD8+) thymocytes, whereas CD4+ single positive thymocytes only are found in the medulla, in keeping with a defect in positive selection of CD8+ T cells.

Genetic counseling

The disease is inherited as a fully penetrant, autosomal recessive trait. Consequently, the risk

for parents of having another child affected is 1:4, regardless of sex of fetus.

Antenatal diagnosis

Although no cases has been diagnosed before birth, antenatal diagnosis can be carried out by molecular analysis of chorionic villus DNA (if the mutation is known) or, later in pregnancy, by fetal blood sampling and analysis of lymphocyte subpopulations and *in vitro* responses to phytohemagglutinin.

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