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Brief Communication

Maternal T-cell engraftment impedes with diagnosis of a SCID-ADA patient

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ABSTRACT

We describe the case of a child affected by severe combined immunodeficiency (SCID) with adenosine deaminase (ADA) deficiency showing a maternal T-cell engraftment, a finding that has never been reported before. The presence of engrafted maternal T cells was misleading. Although ADA enzymatic levels were suggestive of ADA-SCID, the child did not present the classical signs of ADA deficiency; therefore, the initial diagnosis was of a conventional SCID. However, ADA toxic metabolites and molecular characterization confirmed this diagnosis. Polyethylene glycol-modified bovine (PEG) ADA therapy progressively decreased the number of maternal engrafted T cells. The child was grafted with full bone marrow from a matched unrelated donor, after a reduced conditioning regimen, and the result was the complete immunological reconstitution.

1. Introduction

Maternal T cells from fetal or perinatal transplacental passage have been identified in up to 40% of patients with severe combined immunodeficiency (SCID), genetically heterogeneous immune disorders characterized by a dramatic reduction of number and function of T lymphocytes, as well as functional or quantitative defects of B lymphocytes and natural killer (NK) cells [1–3]. While immunocompetent newborns, who have effective T-cell immunity, can rapidly reject the histocompatibility leukocyte antigen (HLA) mismatched maternal lymphocytes, that bidirectionally pass between mother and foetus through the human placenta [4], SCID newborns fail to reject circulating maternal T cells. The maternally derived engrafted T cells, whose number ranges in the blood from 10 to several thousand/ml, are functionally incompetent and show limited or no proliferative response to mitogens. They also usually express a restricted T-cell receptor repertoire [5], a finding suggestive of transplacental passage of a very small number of T cells that successively expand in the host. In the majority of cases, SCID patients with maternal T-cell engraftment are asymptomatic, but approximately 30–40% of them have mild symptoms and signs such as eosinophilia, elevated liver enzymes with periportal T-cell infiltration, and erythema with skin T-cell infiltration. These hepatic and cutaneous manifestations are similar to those observed during the graft versus host disease (GvHD) developing after hematopoietic stem cell transplantation (HSCT) [2]. Maternal T-cell engraftment was not previously described in the best known form of SCID resulting from mutations in the gene encoding for adenosine deaminase (ADA). In ADA-SCID patients, maternal T cells could probably be killed by the raised levels of deoxyadenosine (dAdo), the toxic metabolic substrate accumulated because of the deficient or impaired ADA activity [6]. Affected children usually present with typical clinical and immunological manifestations of SCID patients, together with peculiar features.

Here, we describe a patient with ADA-SCID with maternal T-cell engraftment, which complicated the diagnosis of this rare disease.

2. Material and methods

2.1. Case presentation

A 3.2-month-old Italian girl, born after full-term uneventful pregnancy from non-consanguineous parents, was referred to our hospital with a history of frequent bronchiolitis associated with dermatitis and mycosis.

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2.2. Immunological evaluation

The patient's immunological characterization was performed following the scheme applied in case of a suspected immunodeficiency. Immunophenotyping was performed by a FACSCanto II cytometer combined with the analysis of the data with FACSDiva software (BD Bioscences); lymphocyte proliferation was determined by [^{3}H] thymidine incorporation assay after stimulation with phytohemoagglutinin (PHA), anti–CD3 monoclonal antibody (MoAb), and anti CD3 MoAb plus interleukin-2 (IL-2).

2.3. ADA activity and adenine nucleotide content in red blood cells

ADA activity in red blood cell (RBC) lysates was assayed by spectrophotometrically measuring the rate of ADA absorbance changes at 265 nm. Values were expressed as U/g of Hemoglobin (Hb).

The dAdo nucleotides in RBC (dAXP) were quantified by high performance liquid chromatography measuring the levels of adenosine (Ado) and dAdo formed by treating neutralized acid extracts with alkaline phosphatase and venom phosphodiesterase.

2.4. Maternal engraftment evaluation and quantitative analysis of chimerism

The maternal T-cells engraftment and post-HSCT chimerism status were evaluated using AmpF⁄STR Identifiler Plus PCR Amplification Kit (Life Technologies Inc., Foster City, CA) that, in a short tandem repeat multiplex assay, amplifies 15 tetranucleotide repeat loci and the Amelogenin gender determining marker. The fluorescent PCR products were loaded on a 3130 Genetic Analyzer and the results were analyzed using GeneMapper ID v.3.2 software.

2.5. Analysis of ADA mutation

Amplification of ADA exons from genomic DNA was performed using primers and conditions previously reported [7].

3. Results

Although appearing healthy at birth, at 10 days of life the child developed onychomycosis in both hands and feet that was treated with antibiotics. Thirty days later, she presented skin lesions with impetigo, purulent exudate from the eye, and bronchiolitis. The causative infective agents were not identified. At 2 months of life, the pulmonary function worsened; white blood cells were 5400 cells/μl, neutrophils 4500 cells/μl and lymphocytes 600 cells/μl. Lymphocyte phenotyping revealed a profound CD3 + lymphopenia (189 cells/μl vs 3180–5401 cells/μl in pediatric healthy population [8]), with low CD3 + CD4 + (175 cells/μl vs 2330–3617 cells/μl), CD3 + CD8 + (13 cells/μl vs 712–1361/μl), CD19 + (2 cells/μl vs 315–1383 cells/μl), and NK (47 cells/μl vs 201–870 cells/μl) cell number. IgG were 207 mg/dl (vs 270–1100 mg/dl), IgA were 43 mg/dl (vs 15–110 mg/dl), and IgM were 61 mg/dl (vs 20–170 mg/dl). Clinical symptoms persisted in the following month and lymphopenia worsened: blood cells were 2950 cells/μL, with only 189 CD3 + CD4 + cells/μl. Lymphocyte proliferation to PHA was absent (1×10^3 cpm vs 240×10^3 cpm), reduced to anti–CD3 MoAb (11×10^3 cpm vs 438×10^3 cpm), and to anti-CD3 MoAb plus IL-2 (29×10^3 cpm vs 369×10^3 cpm).

As reported in Table 1, at 3.2 months of life the child showed the typical clinical presentation of SCID and several laboratory findings related to the disease, but she lacked the typical physical and radiological features of ADA-SCID. Therefore, the variable number of tandem repeat analysis revealed that 79.3% of her T lymphocytes were of maternal origin, ADA activity was 0.54 U/g Hb (healthy control range 0.8–2.5 U/g Hb), showing a presence of residual but not absent enzymatic activity [7]. Surprisingly, the values of toxic ADA metabolites were dAXP: 0,629 μmol/ml RBC (healthy control dAXP range < 0.005 μmol/ml RBC), and % dAXP: 28.5 (healthy control % dAXP <0.5).

Table 1
Characteristic of the ADA-SCID patients found in the studied child.

Clinical presentation	Growth failure, absence of lymph nodes and pharyngeal lymph tissue, and characteristic rib abnormalities	Absent
	Infections involving pathogens and opportunistic organisms	present
Radiographic features	Diminished or absent adenoids, cupping/flaring of anterior rib ends, pelvic dysplasia, shortening of vertebral transverse processes, and platyspondylia	absent
Laboratory findings	Very low or undetectable ADA activity	absent
	Elevated toxic ADA metabolites	present
	Lymphopenia	present
	Low or absent in vitro lymphocyte function	present
	Lymphocyte subset defects	present
	Pronounced hypogammaglobulinemia	present
	Lack of maternal engrafted T cells	absent
	ADA sequence variants	present

At 3,4 months of age, a diagnosis of ADA-SCID was made after DNA sequencing, performed with primers designed to amplify all ADA coding exons and the entire 7 and 8 introns [7]. Two mutations were identified in the ADA gene: p.Ser291Leu and p.Leu298Pro; the second mutation not previously described in literature.

Enzyme replacement therapy with 30 U/kg twice weekly of PEG-ADA, together with 200 mg/kg twice monthly of intravenous immunoglobulins was immediately initiated after the diagnosis. After this therapy, engrafted maternal T cells of child dropped from 79.3% to 9.2%, and toxic ADA metabolites decreased (dAXP: 0.008 μmol/ml RBC, and % of dAXP: 0.429) within 5 months.

Finally at 11 months of life, after the recovery from a coronavirus infection diagnosticated at 6 months, she underwent HSCT from a matched unrelated donor (MUD, 10/10 HLA-matched), since an HLA-identical family donor was not available. The patient received a reduced-intensity myeloablative conditioning [150 mg/m^2 fludarabine, 36 g/m^2 treosulfan, and anti thymocyte globulin (ATG)]. Infused cells were $14{,}29 \times 10^6$ CD34 +/Kg and $69{,}47 \times 10^6$ CD3 +/Kg. Platelet and neutrophil engraftments were achieved 16 and 17 days after HSCT, respectively. A complete donor chimerism was detected in lymphocytes and polymorphonucleated cells 20 days after HSCT. Lymphocyte proliferation to mitogens normalized after 5.1 months post HSCT (PHA: 51.5 $\times 10^3$ cpm, anti–CD3 MoAb 320.6 $\times 10^3$ cpm, and anti-CD3 MoAb plus IL-2: 251.5 $\times 10^3$ cpm), while, due to the use of ATG as part of the conditioning, full immunological reconstitution was achieved by 9.6 months post HSCT (CD3 +: 1403 cells/μl, CD3 + CD4 +: 864 cells/μl, CD3 + CD8 +: 424 cells/μl, CD19 +: 381 cells/μl, and NK 61 cells/μl).

Now the child is 26 months old; she is in good health, and without signs of GvHD.

4. Discussion

SCID are diseases difficult to be recognized. In infants, early-onset severe respiratory tract infections, infectious diseases caused by opportunistic pathogens, chronic diarrhea and failure to thrive are the common presentation picture of a child affected by SCID [3]. The severity of clinical presentation depends on how compromised is the immune system. Maternal engraftment is a common feature in SCID patients; engrafted T lymphocytes contain a high fraction of functional regulatory T cells, conferring a high-tolerance capacity that appears to represent a toleration advantage beside the severe immunodeficiency. Maternal cells could provided some degree of immunity and, therefore, may protect the child from severe infections [5].

It was commonly accepted that the presence of large amounts of maternal T cells in SCID patients can only occurs if host T cells are lacking. This thought was disproved by the recent findings of long-term coexistence of engrafted maternal T cells and autologous T cells in a SCID

patient with a Janus kinase (JAK) 3 mutation [9] and in a patient with mild Omenn's Syndrome phenotype [5]. In rare cases, engrafted maternal T cell might persist for long time leading to partial reconstitution of immune function and delayed clinical presentation of SCID [10]. In our contest maternal engraftment test and enzymatic ADA assay were performed, to better define the disease. The presence of maternal engrafted T cells and residual ADA activity enzyme of our patient could have delayed the identification of ADA-SCID. The laboratory tests showed low number of CD3 + cells but toxic ADA metabolites suggested the diagnosis of ADA-SCID. The molecular analysis of ADA gene allowed the conclusive diagnosis.

In this disease, ADA metabolites have toxic effects on different organ systems, most notably in the immune system. It is still not entirely clear, however, whether lymphocytes from ADA-deficient patients are intrinsically abnormal or whether the observed defects are secondary to the effect of deoxyadenosine triphosphate and dAdo accumulation. Therefore in our patient ADA metabolites hadn't have a clear toxic effect on T lymphocytes of maternal origin.

In conclusion we described a case of maternal engrafted T cells in an infant with ADA-SCID, a type of immunodeficiency in which the engrafted T cells were never described before. The patient did not show all the typical early-onset ADA-deficient SCID symptoms, so that only the molecular analysis of ADA gene allowed the disease diagnosis.

Therefore fast correct diagnosis is crucial for an early treatment; on the contrary, the expectancy of life for these children would be 1–2 years. Moreover, HSCT has a very high rate of cure, depending on the infectious status before transplant and the age of the patient at transplant, with the youngest going better. Accordingly, in our patient the early HSCT had provided a definitive treatment. Although post-HSCT acute GvHD developed significantly more in SCID patients with maternally engrafted T cells [11], our patients did not show this complication.

Ethical approval

This study was approved by the Comitato Etico di Brescia (protocol number NP 0)

Informed consent

Informed consent was obtained from parents of the ADA-SCID patient.

Conflict of interest disclosure

The authors declare that they have no conflict of interest.

Authorship contributions

All authors have made substantial contributions to all of the following: (1) the conception and design of the study, acquisition of data, analysis and interpretation of data, (2) drafting the article and revising it critically for important intellectual content, (3) final approval of the version.

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