

Letter to the Editor

Anhidrotic ectodermal dysplasia: A new mutation

To the Editor:

Hyper-IgM syndrome is a genetically heterogeneous clinical condition, characterized by high/normal levels of IgM with low/absent levels of IgA, IgG, and IgE, indicating a defect in class-switch recombination. Hyper-IgM immunodeficiency may be associated with the anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID), caused by mutations in the gene encoding either nuclear factor kappa B (NF- κ B) essential modulator (*NEMO*)/I κ B kinase γ (*IKK γ*) (X-linked-EDA-ID) or the inhibitor of NF- κ B (*I κ B*) regulated by the IKK complex, which includes I κ B α , I κ B β , and I κ B ϵ (autosomal-EDA-ID).^{1,2} We describe an infant with EDA-ID caused by a new mutation of *I κ B α* , not yet reported in the literature.

A 5-month-old female infant born to unrelated parents, sparse-haired and with evident superficial cutaneous *venous reticulum* on her dry and thin skin, was hospitalized for bilateral bronchopneumonia, fever, and suspected septicemia resistant to antibiotic and steroid therapy. She presented hypogammaglobulinemia (IgG 0.3 g/L, IgA <0.05 g/L) with increasing value of IgM (7 g/L), absence of allohemagglutinin titers, and no antibody response to tetanus toxoid and *Haemophilus influenzae* (Table I). So, substitutive therapy with intravenous immunoglobulins (400-800 mg/kg/month) was administered with little effect: in the period between age 6 and 12 months, she developed severe respiratory failure, chronic diarrhea, vomiting, loss of appetite and fever, with increased inflammation indexes, followed by 2 episodes of sepsis by opportunistic organisms, respectively, *Stenotrophomonas maltophilia* and *Candida albicans*, diffuse impetigo, and osteomyelitis of skull and limbs. Three consecutive sweat tests

failed for hypohidrosis, while the other metabolic investigations all resulted as normal. The sudden worsening of clinical conditions stopped us from carrying out the planned stem cell transplant, and the patient was placed in a laminar-flow room. Despite this measure, the infant first developed wide oral candidiasis involving the esophagus, then sepsis by *Klebsiella pneumoniae*, and finally sepsis by *Candida parapsilosis*, complicated with fatal disseminated intravascular coagulation. During the hospitalization, we performed immunologic and genetic studies.

At the age of 5 months, T cells were present at the expected frequency (57% of the cells in the lymphocyte gate) and the ratio CD4/CD8 was normal. The frequency of CD45ROpos memory B cells was low-normal for the age (5% of T lymphocytes). T-cell proliferation to anti-CD3-CD28, evaluated by 5-chloromethylfluorescein diacetate, was normal. Both CD4 and CD8 T cells proliferated (Fig 1), and no significant alteration was found after stimulation with PHA and phorbol 12-myristate 13-acetate plus ionomycin (Table I).

B cells were 40% of the lymphocytes, and all had the transitional B-cell phenotype. In normal neonates, transitional B cells are the largest population, but rare memory B cells mostly expressing IgM are present at age 5 months. This population that represents around 5% of B cells in the neonatal controls was absent in the patient. We also measured the *in vitro* response to CpG. In the control, as expected, 56% of the B cells proliferated and differentiated into plasma cells, producing antibodies, mostly of the IgM isotype. The patient's B cells showed a reduced survival *in vitro*, and the response to CpG was markedly reduced (15%) (Fig 1).

Molecular investigations showed normal *CXCR4*, *CD40*, and *AICDA*, and so we excluded the WHIM (warts,

TABLE I. Immunologic characteristics of the patient

Age at evaluation	Lymphocyte flow cytometry (absolute values)					Mitogen stimulation	Immunoglobulins (g/L)			Vaccine titers	
	CD3	CD4	CD8	CD16/56	CD19/20		IgG	IgA	IgM	Tetanus (IU/mL)	Hib (mg/L)
6 mo	15008	7616	5824	224	7168	Low response of B cells stimulated with CpG (2 samples)	0.3	0.11	3.04		
7 mo							0.3	0.05	7		
8 mo*						Normal response of T cells with anti-CD3+CD28+; no alteration of proliferative response after PMA and PHA (2 samples)	2.1	0.6	2.33		
10 mo*	14268	9184	5084	328	1476	Low response of B cells stimulated with CpG (2 samples)	5.2	0.09	1.77	0.05	0.1
12 mo							4.4	0.13	1.02		
13 mo	21080	12896	7936	992	2480	Normal response of T cell with anti-CD3+CD28; no significant alteration of proliferative response after PMA and PHA (2 samples)	5.1	0.23	0.95		
14 mo*							10.9	0.33	1.9		

Hib, *Haemophilus influenzae b*; PMA, phorbol 12-myristate 13-acetate plus ionomycin.

*After intravenous immunoglobulins (IVIGs).

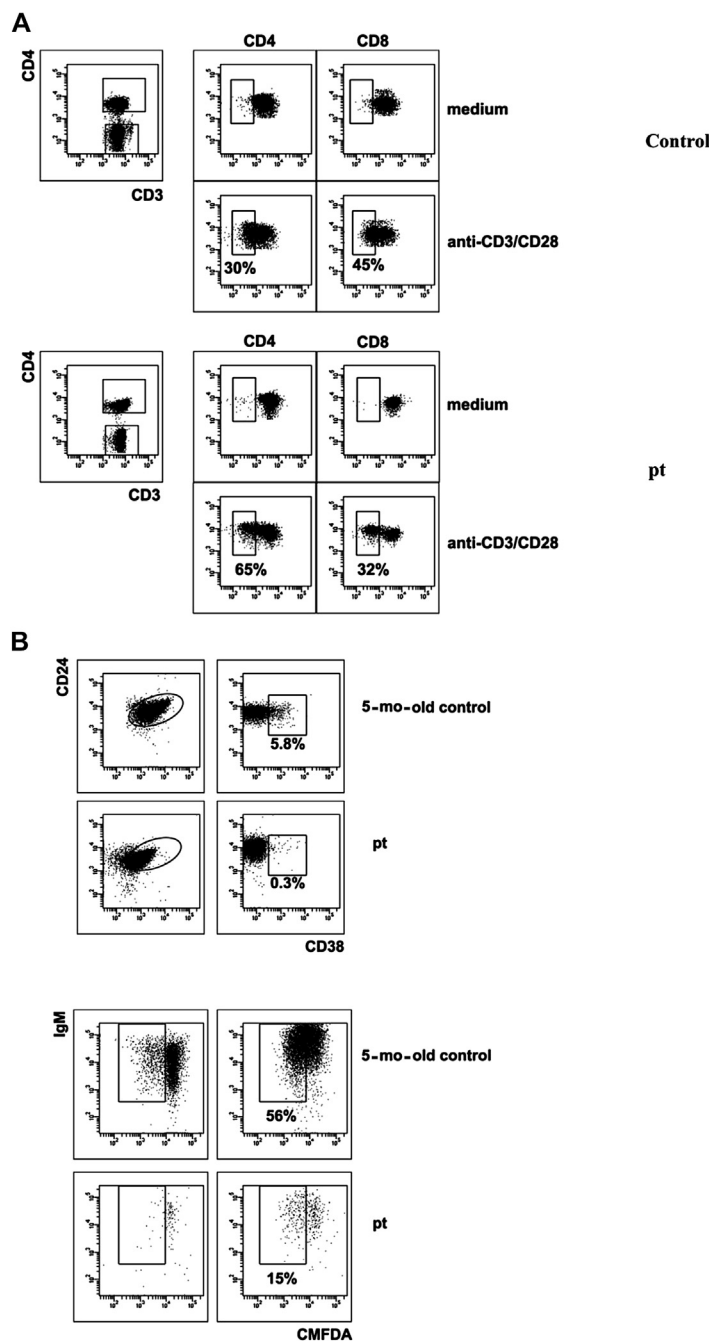


FIG 1. T-lymphocyte proliferation (**A**) and B-lymphocyte response to CpG (**B**) in our patient versus control. *pt*, Patient.

hypogammaglobulinemia, infections and myelokathexis) syndrome and the classic form of hyper-IgM syndrome.¹ Because the patient was female, her medical history and phenotype induced us to perform the analysis of exons 1 to 6 and the corresponding exon/intron junctions of the gene encoding the I κ B α protein, previously described to cause an autosomal-dominant form of EDA and T-cell immunodeficiency in few patients.³⁻⁷ We identified 7 polymorphisms already described in the literature and a *de novo* heterozygous mutation in exon 1, consisting in a T to G substitution of the nucleotide 110 of the cDNA of the gene (NM_020529: c.T110G). This causes the change of methionine

(M) at position 37 with an arginine (R) (p.M37R). This protein maps to the conserved amino acid residue adjacent to Ser36, 1 of the 2 phosphorylation sites that are essential for targeting I κ B α for proteasomal degradation and activation of NF- κ B, and is predicted to be damaging by PolyPhen-2. The M37R mutation was not found at <http://www.1000genomes.org> and <http://evs.gs.washington.edu/EVS/> databases. In addition, we sequenced 192 control chromosomes and we did not find it. Recently, a heterozygous mutation in the same residue has been described in a boy presenting with EDA-ID and the authors demonstrated that the mutation impairs nuclear translocation of NF- κ B in patient

fibroblasts.⁸ All together, all these pieces of evidence strongly support the pathogenicity of the M37R missense mutation.

Indeed, patients with ectodermal dysplasia present with impaired development of sweat glands in their skin and this is an important indication, together with recurrent infections, to suspect a role of NF- κ B signaling.^{5,6} The most common mutation of ectodermal dysplasia with immunodeficiency is a hypomorphic X-linked recessive mutation in the gene encoding NEMO protein-IKK γ , the regulatory subunit of the IKK complex, which also includes the kinases IKK α and IKK β . When NEMO protein is altered, phosphorylation of key serine residues of the NF- κ B inhibitor, I κ B, and its consequent degradation are impaired. This does not allow NF- κ B to enter the nucleus to regulate gene transcription. As a result, these subjects have a notable susceptibility to infections, especially those caused by pyogenic bacteria and mycobacteria.^{2,7,9} A common autosomal-dominant form of EDA-ID is associated with a heterozygous missense mutation at serine 32 of I κ B α , a subunit of I κ B, altering NF- κ B activation. NEMO and I κ B α mutations largely overlap and include the same characteristics of EDA-ID, significantly reducing the normal cellular responses to proinflammatory cytokines (TNF- α and IL-1) and Toll-like receptor ligands. However, the autosomal-dominant form of EDA-ID, but not the X-linked form of EDA-ID is associated with a severe and unique T-cell immunodeficiency. The main immunologic abnormalities are usually represented by a marked blood lymphocytosis with no detectable memory T cells *in vivo* and naïve T cells not responding to CD3-TCR activation *in vitro*.^{4,7,9} However, the immunologic phenotype may be heterogeneous, with many cases presenting a normal number of T cells, B cells, and natural killer cells, as well as a variable lymphocyte proliferation in response to different antigens,^{5,6} as in our patient. IgG levels in patients with EDA-ID are commonly reduced with increased IgM or IgA level and impaired specific antibody responses to protein and polysaccharide antigens.^{1,6} The immunologic heterogeneity and the knowledge of different forms of EDA-ID (X-linked for NEMO mutations and autosomal dominant due to I κ B α gene mutations) suggest that other components of the NF- κ B activation pathway, in addition to NEMO and I κ B α , can be mutated in humans, generating an EDA-ID-related disease.³ We report, for the first time, a novel I κ B α gene mutation: the patient's history, the clinical presentation, the immunologic phenotype, and the mutated gene strongly suggest the causative role of the mutation discovered and show that when such conditions are present, NF- κ B function should be evaluated for mutations in the NF- κ B pathway.⁹ This could provide novel targets for therapeutic intervention in diseases characterized by dysregulated NF- κ B

activation. Today, the only resolutive therapy for EDA with T-cell immunodeficiency is stem cell transplant, which unfortunately was not possible in our patient because of the rapid course of her disease.¹⁰

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