Cernunnos Deficiency: A Case Report

T Turul, I Tezcan, O Sanal

Hacettepe University Medical Faculty Department of Pediatrics, Immunology Unit, Ankara, Turkey

Abstract

B cell–negative severe combined immunodeficiency (SCID) is caused by molecules involved in the variable (diversity) joining (V[D]J) recombination process. Four genes involved in the nonhomologous end joining pathway—*Artemis*, *DNA-PKcs*, *DNA ligase 4*, and *Cernunnos*—are involved in B cell–negative radiosensitive SCID. Deficiencies in *DNA ligase 4* and the recently described *Cernunnos* gene result in microcephaly, growth retardation, and typical bird-like facies. Lymphopenia and hypogammaglobulinemia with normal or elevated immunoglobulin (Ig) M levels indicate a defect in V(D)J recombination. We present a case with recurrent postnatal pulmonary infections leading to chronic lung disease, disseminated molluscum contagiosum, lymphopenia, low IgG, IgA and normal IgM levels. Our patient had phenotypic features such as microcephaly and severe growth retardation. Clinical presentation in patients with the B cell–negative subtype ranges from SCID to atypical combined immunodeficiency, occasionally associated with autoimmune manifestations and cytomegalovirus infection. Our patient survived beyond infancy with combined immunodeficiency and no autoimmune manifestations.

Key words: Cernunnos. Immunodeficiency. Syndrome.

Resumen

La inmunodeficiencia combinada grave (IDCG) negativa para linfocitos B está causada por moléculas que participan en el proceso de recombinación de los segmentos variable, de diversidad y de unión (V[D]J). En la IDCG negativa para linfocitos B radiosensible están implicados cuatro genes (*Artemis, DNA-PKcs, ADN ligasa IV* y *Cernunnos*) que participan en la vía de recombinación no homóloga. Las deficiencias en el gen *ADN ligasa IV* y en el gen *Cernunnos* recientemente descrito dan lugar a microcefalia, retraso del crecimiento y facies de pájaro típica. La linfopenia y la hipogammaglobulinemia con niveles normales o elevados de inmunoglobulina (Ig) M indican un defecto en la recombinación V(D)J. En este trabajo se presenta un caso con infecciones pulmonares posnatales recurrentes derivando en enfermedad pulmonar crónica, molusco contagioso diseminado, linfopenia y síndrome de hiper-IgM. El paciente presentaba características fenotípicas como microcefalia y retraso grave del crecimiento. La presentación clínica en pacientes con el subtipo negativo para linfocitos B oscila entre la IDCG y la inmunodeficiencia combinada atípica, en ocasiones asociada a manifestaciones autoinmunitarias e infección por citomegalovirus. El paciente sobrevivió más allá del primer año de vida con inmunodeficiencia combinada y sin manifestaciones autoinmunitarias.

Palabras clave: Cernunnos. Inmunodeficiencia. Síndrome.

Introduction

Severe combined immunodeficiency (SCID) is an inherited group of diseases in which both cellular immunity and humoral immunity are defective. Depending on the degree of the defect in lymphoid differentiation—either complete absence of T and B cells or blockage of T lymphocyte development—2 groups of SCID have been defined: B cell–negative SCID and B cell–positive SCID [1]. Efforts to clarify the underlying molecular defects of the former phenotype, which accounts for approximately 35% of all cases of SCID, revealed 4 genes involved in the nonhomologous end joining (NHEJ) pathway, which is the major repair mechanism in response to double strand breaks [2]. The 4 genes are *Artemis*, *DNA-PKcs*, *DNA ligase 4*, and *Cernunnos* (or XRCC4-like factor [XLF]). In addition to radiosensitivity, which is common to all 4, defects in *DNA ligase 4* and *Cernunnos* lead to microcephaly and growth retardation [2].

We present a patient with B cell–negative SCID, microcephaly, and growth retardation due to a defect in the *Cernunnos* gene.

Case Description

The patient was a female infant of consanguineous parents who had failed to thrive and suffered wheezing attacks requiring admission to hospital since 6 months of age. She was admitted for pulmonary infection at 6 months and 19 months, although the etiologic agent and treatment were not recorded during these episodes. She had intermittent mucocutaneous candidiasis that responded to therapy. On admission at 20 months of age, her physical examination revealed rales and rhonchi on pulmonary auscultation, microcephaly (head circumference was 40 cm, below the first percentile), clubbing, and weight and height below the fifth percentile. The family history revealed death of 3 female infant siblings due to infections. The immunological assessment revealed low levels of immunoglobulin (Ig) G and IgA hypogammaglobulinemia with normal IgM levels, lymphopenia, low T- and B-cell counts, and significant reduction in lymphocyte proliferative responses (Table). Monitoring of Ig levels showed panhypogammaglobulinemia, as IgM levels were also decreased over time. Antibacterial and antifungal prophylaxis and monthly intravenous immunoglobulin replacement therapy were initiated. Mutation analysis revealed a homozygous *C622T* nonsense mutation in exon 5 that changed an arginine codon at position 178 to a stop codon (R178X). Despite receiving intravenous immunoglobulin, the patient continued to have recurrent pulmonary infections and purulent otitis due to *Haemophilus influenza* and *Streptococcus pneumoniae* (treated with ampicillin-sulbactam, clarithromycin, and amikacin). She had wheezing attacks at 21 months, 2 years, and 3 years of age requiring hospitalization. High-resolution computed tomography revealed the presence of bronchiolitis obliterans at the age of 3.5 years, and she developed pulmonary hypertension secondary to chronic lung disease. At 4 years of age, widespread molluscum contagiosum developed around the neck and on the chest. Her head circumference was 42 cm (below the first percentile). The patient died suddenly at home of unknown causes at 4.5 years of age.

Table. Immunological Parametersa

	Ferrous Sulphate	
Absolute lymphocyte counts, x10^6/μL)	700-1120 (2700-11 900)	
T cells ($CD3^+$)	132-750 (1400-8000)	
$CD3^+CD4^+$	99-380 (900-5500)	
$CD3^+CD8^+$	316-436 (400-2300)	
B cells	67-56 (600-3100)	
NK cells	257-316 (100-1400)	
Serum Ig levels, ng/dL		
IgA	4.6-11.7 (11-4)	
IgG	17-561 (633-1466)	
IgM	33-42 (22-87)	
In vitro lymphocyte proliferation test, cpm×10–3		
	Patient	Control
SI_{PHA}	21.5/5.9	105.4/157.5
SI_{ConA}	34.1/18.2	135.8/9.6
$SI_{antiCD3}$	21.2/18.1	100.3/62.8
SI_{PMA+I}	25.1/–	145/–

Abbreviations: Ig, immunoglobulin; SI, stimulation index.
[a]Numbers in parenthesis indicate the reference range.

Discussion

We describe a patient with microcephaly, growth retardation, recurrent infections, lymphopenia, and IgG and IgA hypogammaglobulinemia with normal IgM levels. An underlying molecular defect was shown in the *Cernunnos* gene, which was recently identified as the responsible gene in patients with B cell–negative radiosensitive (RS) SCID in whom *DNA ligase* 4 deficiency was ruled out.

Human SCID can arise when DNA repair via the NHEJ pathway is defective. Patients with B cell–negative SCID exhibit radiosensitivity. NHEJ is a predominant pathway of DNA double strand break repair in mammalian cells; defects in this pathway cause radiosensitivity at the cellular level. NHEJ defects in animals are also usually associated with defective V(D)J recombination and/or immunoglobulin class-switch recombination, leading to SCID or related immunodeficiencies [3]. V(D)J recombination is a somatic rearrangement of Ig and T-cell receptor genes in order to create a diversity of lymphocyte antigen receptors. V(D)J recombination takes place during somatic hypermutation and class switch recombination (both crucial for development and maturation of T and B lymphocytes). The double strand breaks formed by the recombination activating gene (*RAG1*) and *RAG2*, which are specific to lymphocytes, are repaired by NHEJ proteins. The 3'OH at each nick attacks the antiparallel strand to form hairpin intermediates. Ku proteins bind to the DNA ends, and the Artemis-DNA dependent protein kinase-catalytic subunit (DNA-PKcs) complex, which is recruited to the DNA ends by Ku, opens the hairpins. The x-ray repair cross complementing protein 4 (XRCC4)-DNA ligase 4 complex ligates the DNA ends. Cernunnos promotes NHEJ in part by regulating the activity of XRCC4-DNA ligase 4 complex [3]. Among the 7 genes defined, deficiencies in 4—*Artemis*, *DNA-PKcs*, *DNA ligase 4*, and *Cernunnos* (XLF)—have been shown to result in B cell–negative SCID. Radiosensitivity is common to all 4 genes [4-6]. V(D)J recombination is affected in all defects of these genes, and this in turn affects lymphoid development and maturation.

Although null mutations of the *Artemis* gene result in a classic B cell–negative SCID phenotype, hypomorphic mutations of the same gene may cause either Omenn syndrome or progressive combined immunodeficiency in later infancy characterized by T and B lymphopenia, hypogammaglobulinemia, and autoimmune cytopenia [7,8]. However, microcephaly and growth retardation are not associated with clinical phenotypes of defects in *Artemis* and *DNA-PKcs* [6-8]. In contrast, defects in the other 2 genes, *DNA ligase 4* and *Cernunnos*, cause a similar phenotype with growth retardation and microcephaly.

Cernunnos deficiency has recently been described by 2 independent groups [3,5]. *Cernunnos* interacts with the XRCC4-DNA ligase 4 complex, which has a central role in

NHEJ. To date, 8 cases of *Cernunnos*-deficient combined immunodeficiency and low T and B cell counts have been reported. The first 2 had T–B–NK+ SCID with radiosensitivity and the remaining 6 had mild combined immunodeficiency with low IgA and IgG levels. In 2 of the patients, IgM levels were elevated, indicating impairment in the class-switch recombination process [2-5,9]. All had recurrent bacterial and opportunistic infections, whereas some had autoimmune cytopenia. Microcephaly was present in some of the cases, but all the patients had severe growth retardation. This clinical phenotype closely resembles DNA ligase 4 deficiency, in which the clinical spectrum can vary from an almost normal immune system to T–B–NK+ radiosensitive SCID [10-12].

Ineffective V(D)J recombination is responsible for immunodeficiency in patients with B cell–negative radiosensitive SCID. V(D)J recombination is severely defective in *Artemis*-deficient radiosensitive SCID patients in whom T- and B-cell counts are also significantly reduced. In contrast, V(D)J deficiency is less severe than *Artemis*-deficient radiosensitive SCID. In addition, in both *Cernunnos* deficiency and *DNA ligase 4* deficiency, in vitro findings regarding V(D)J recombination deficiency are less severe than the clinical immunodeficiency observed [5,13]. In the only reported case due to *DNA-PKcs* deficiency, both in vitro and clinical findings were similar to *Artemis* deficiency [6]. Therefore it seems that *Cernunnos* has essential functions during lymphocyte development other than its involvement in V(D)J recombination [2].

In the present case, whose *Cernunnos* deficiency was confirmed and reported elsewhere [5], the clinical presentation was that of combined immunodeficiency. The principal laboratory findings were T and B lymphopenia accompanying IgG and IgA hypogammaglobulinemia despite normal levels of IgM and low in vitro lymphocyte proliferative responses. The Ig pattern points to a deficiency in class-switched recombination in addition to defective V(D)J recombination. Phenotypic findings such as microcephaly and proportional growth retardation were present in our case. Microcephaly in these patients might suggest a role for the *Cernunnos* gene during the development of the central nervous system [5]. Autoimmunity has also been reported in patients with *Cernunnos* deficiency, as in other combined immunodeficiencies with low T- and B-cell counts, such as hypomorphic *RAG1* mutation. This may be the consequence of defective helper T cells and/or reduced B-cell repertoire diversity in the setting of a defective but not fully abrogated V(D)J rearrangement [14]. The present case does not have autoimmune manifestations. However, we have 2 additional patients with *Cernunnos* deficiency who underwent successful bone marrow transplantation (submitted for publication). Both had lymphopenia and low IgG and IgA levels and phenotypic features such as microcephaly and growth retardation. One, who is the cousin of the present case, presented with SCID and received bone marrow from her HLA-identical sibling in early infancy. The other, diagnosed at 4 months of age, had recurrent lower respiratory tract infections since 2 months of age accompanied by autoimmune hemolytic anemia and cytomegalovirus infection. She also benefited from HLA-identical bone marrow transplantation.

Other potential molecular defects in radiosensitive SCID (eg, Ku70/80, XRCC4, and other factors playing a role in NHEJ) have not yet been described. Performing mutation analysis in combined immunodeficiency patients with low T- and B-cell counts, autoimmune cytopenia, growth retardation, and microcephaly will make it possible to identify new defects and to better define genotype–phenotype correlation and treatment options.

References

1. Geha RS, Notarangelo LD, Casanova JL, Chapel H, Conley ME, Fischer A, Hammarström L, Nonoyama S, Ochs HD, Puck JM, Roifman C, Seger R, Wedgwood J, International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. Primary immunodeficiency diseases: An update from the International Union of Immunological Societies for Primary Immunodeficiency Diseases Classification Committee. J Allergy Clin Immunol. 2007;120:776-94.
2. Schwarz K, Ma Y, Pannicke U, Lieber MR. Human severe combined immunodeficiency and DNA repair. BioEssays. 2003;25(11):1061-70.
3. Ahnesorg P, Smith P, Jackson SP. XLF interacts with XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. Cell. 2006;124:301-13.
4. Gennery AR. Primary immunodeficiency syndromes associated with defective double stranded-break repair. Br Med Bull. 2006;77-78:71-85.
5. Buck D, Malivert L, De Chasseval R, Barraud A, Fondaneche MC, Sanal O, Plebani A, Stephan JL, Hufnagel M, Le Deist F, Fischer A, Durandy A, de Villartay JP, Revy P. Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. Cell. 2006;124:287-99.
6. Van der Burg M, Ijpeerts H, Verkaik NS, Turul T, Wiegant WW, Morotomiyano K, Mari PO, Tezcan I, Chen DJ, Zdzienicka MZ, van Dongen JJ, van Gent DJ. A DNA-PKcs mutation in a radiosensitive T-B-SCID patient inhibits Artemis activation and nonhomologous end-joining. J Clin Invest. 2009;119(1):91-8.
7. Mochous D, Callebaut I, de Chasseval R, Poinsignon C, Villey I, Fischer A, de Villartay JP. Artemis, a novel DNA double-strand break repair / V(D)J recombination protein, is mutated in severe combined immunodeficiency. Cell. 2001;105:177-86.
8. Ege M, Ma Y, Manfras B, Kalwak K, Lu H, Lieber MR, Schwarz K, Pannicke U. Omenn syndrome due to ARTEMIS mutations. Blood. 2005;105:4179-86.
9. Dai Y, Kysela B, Hanakahi LH, Manolis K, Riballo E, Stumm M, Harville TO, West SC, Oettinger MA, Jeggo PA. Nonhomologous end-joining and V(D)J recombination require an additional factor. Proc Natl Acad Sci USA. 2003;100:2462-7.
10. O'Driscoll M, Cerosaletti KM, Girard PM, Dai Y, Stumm M, Kysela B, Hirsch B, Gennery A, Palmer SE, Seidel J, Gatti RA, Varon R, Oettinger MA, Neitzel H, Jeggo PA, Concannon P. DNA ligase 4 mutations identified in patients exhibiting developmental delay and immunodeficiency. Mol Cell. 2001;8:1175-85.
11. van der Burg M, van Veleen VR, Verkaik NS, Wiegant WW, Hartwig NG, Barendregt BH, Brugmans L, Raams A, Jaspers NG, Zdzienicka MZ, van Dongen JJ, van Gent DC. A new type of radiosensitive T-B-NK+ severe combined immunodeficiency caused by a LIG 4 mutation. J Clin Invest. 2006;116:137-45.

12. Buck D, Moshous D, de Chasseval R, Ma Y, Le Deist F, Cavazzano-Calvo M, Fischer A, Casanova JL, Lieber MR, de Villartay JP. Severe combined immunodeficiency and microcephaly in siblings with hypomorphic mutations in DNA ligase 4. Eur J Immunol. 2006;36:224-35.
13. Villartay de JP, Lim A, Al-Mousa H, Dupont S, Dechanet-Merville J, Coumau-Gatbois E, Gougeon M-L, Lemainque A, Eidenschenk C, Jouanguy E, Abel L, Casanova JL, Fischer A, Le Deist F. A novel immunodeficiency associated with hypomorphic RAG1 mutations and CMV infection. J Clin Invest. 2005;115:3291-9.

Manuscript received August 3, 2010; accepted for publication November 26, 2010.

Özden Sanal, MD

Hacettepe University Medical
Faculty Department of Pediatrics
Immunology Unit
06100, Sıhhiye, Ankara, Turkey
E-mail: sanaloz@tr.net