CD27 deficiency is associated with combined immunodeficiency and persistent symptomatic EBV viremia

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Background: CD27 is a lymphocyte costimulatory molecule that regulates T-cell, natural killer (NK) cell, B-cell, and plasma cell function, survival, and differentiation. On the basis of its function and expression pattern, we considered *CD27* a candidate gene in patients with hypogammaglobulinemia.
Objective: We sought to describe the clinical and immunologic phenotypes of patients with genetic CD27 deficiency.
Methods: A molecular and extended immunologic analysis was performed on 2 patients lacking CD27 expression.
Results: We identified 2 brothers with a homozygous mutation in *CD27* leading to absence of CD27 expression. Both patients had persistent symptomatic EBV viremia. The index patient was hypogammaglobulinemic, and immunoglobulin replacement therapy was initiated. His brother had aplastic anemia in the course of his EBV infection and died from fulminant gram-positive bacterial sepsis. Immunologically, lack of CD27 expression was associated with impaired T cell–dependent B-cell responses and T-cell dysfunction.
Conclusion: Our findings identify a role for CD27 in human subjects and suggest that this deficiency can explain particular cases of persistent symptomatic EBV viremia with hypogammaglobulinemia and impaired T cell–dependent antibody generation. (J Allergy Clin Immunol 2012;129:787-93.)

Key words: *EBV, viremia, hypogammaglobulinemia, CD27, immunodeficiency, T cell, B cell, natural killer cell, phenotype*

Abbreviations used
CTL: Cytotoxic T lymphocyte
CVID: Common variable immunodeficiency
FACS: Fluorescence-activated cell sorting
GC: Germinal center
NK: Natural killer
PWM: Pokeweed mitogen
SAC: *Staphylococcus aureus* Cowan I antigen
TT: Tetanus toxoid

CD27, a member of the TNF receptor family, is a transmembrane receptor that is widely used as a leukocyte differentiation marker for subsets of T, natural killer (NK), and B cells.[1-3] Importantly CD27 is recognized as a marker for memory B cells and is held to be of diagnostic/predictive value in patients with common variable immunodeficiency (CVID). In patients with CVID, there can be decreased numbers of switched memory B cells (expressing surface IgA or IgG), which correlates with the presence of splenomegaly and granulomatous disease.[4]

The function of human and murine CD27 has been studied in detail *in vitro* and *in vivo* in murine models.[5,6] Ligation of CD27 by its unique ligand CD70 provides costimulatory signals for T-, B-, and NK cell activation. It furthermore enhances T-cell survival and effector function, NK cell function, B-cell differentiation, and plasma cell function.[7,8] In human subjects an indispensable role of the costimulating signal provided by CD27-CD70 interaction toward immune function and disease susceptibility has not been formally proved. CD27 is a major differentiation/maturation marker for T cells, NK cells[9] and B cells.[10] On the basis of its expression on memory B cells and plasma cells, and the effect of CD27 ligation on *in vitro* B-cell function, *CD27* has been proposed as a candidate gene in patients with CVID, but its expression on B, T, and NK cells suggests that CD27 deficiency might result in a more combined type of immune deficiency.

Primary EBV infection is often asymptomatic in the immunocompetent host. In immunodeficient patients, however, primary EBV infection or secondary reactivation might result in persistent symptomatic EBV viremia, a clinical condition with a prolonged (>6 months) and distinct symptomatic phase with fever, lymphadenopathy, and several other possible features, such as hepatitis and pneumonia. Persistent symptomatic EBV viremia can be associated with lymphoma, lymphoproliferative disease, hemophagocytic lymphohistiocytosis, and aplastic anemia but most typically goes into spontaneous remission.[11]

EBV-specific immunity typically encompasses virus-specific cellular and humoral immune responses, with T cells being most important for long-term control of disease. Several types of cellular immune deficiency can result in an abnormal course of EBV infection, including combined immune deficiencies, X-linked

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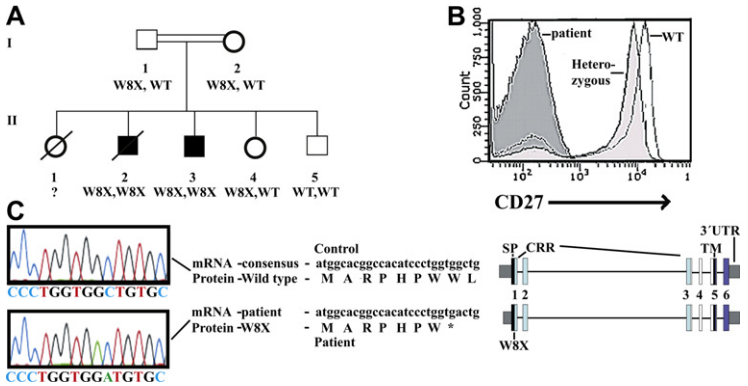

FIG 1. Absence of CD27 expression because of a nonsense mutation in the *CD27* gene. **A,** Family pedigree (/, deceased persons; *double lining*, consanguinity). **B,** FACS analysis of CD27 membrane expression on PBMCs. **C,** Sequence analysis of the *CD27* gene. The mutation *(boldface)* identified in both patients in exon 1 results in a premature stop codon *(X)* in the signal peptide of the protein (NP_001233.1), as depicted in the protein sequence (superimposed on the gene sequence).

lymphoproliferative disease,[12] familial hemophagocytic lymphohistiocytosis,[13] and IL-2–inducible T-cell kinase deficiency.[14] In the majority of persistent symptomatic EBV viremia cases, however, a specific primary immune deficiency has not been identified.

We here describe 2 brothers with CD27 deficiency caused by a homozygous mutation resulting in a premature stop codon in the gene encoding CD27. Clinically, these patients presented as having persistent symptomatic EBV viremia, with lethal aplastic anemia in one and hypogammaglobulinemia with impaired specific antibody function in the other. In the surviving patient the absence of CD27 was associated with an abnormal T cell–dependent B-cell response and disturbed T-cell function.

METHODS

Evaluation of blood, bone marrow biopsy specimens, vaccination responses, and medical records were carried out after written informed consent was obtained in accordance with local medical ethics committee guidelines.

Case report

The index patient, a 21-year-old man of Moroccan descent, was the third child of consanguineous parents (first cousins). At age 2½ years, he experienced fever, severe lymphadenopathy, and hepatosplenomegaly lasting a total of 6 months. EBV seroconversion was noted for early antigen and viral capsid antigen, but during follow-up, no seroconversion for Epstein-Barr nuclear antigen was noted. Immunoglobulin levels were determined longitudinally and were initially increased (IgM, 3.1 g/L; IgG, 15.9 g/L; and IgA, 1.9 g/L; see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). The peripheral blood lymphocyte compartment was phenotyped regularly during the first 1½ years of follow-up, and changes in lymphocyte numbers showed signs compatible with viral infection (see Fig E1, *A*). T-cell proliferation assays showed strongly reduced mitogen- and antigen-specific responses the first 6 months after clinical presentation, and these responses gradually increased to subnormal and normal levels, respectively, during the following year (data not shown). Clinical symptoms disappeared after 6 months, and at the same time, the index patient became hypogammaglobulinemic (IgM, 0.03 g/L; IgG, 4.4 g/L; and IgA, 0.1 g/L). Immunoglobulin replacement therapy was initiated. Since receiving immunoglobulin prophylaxis, he has had an uneventful medical history: no abnormalities were noted in the incidence, type, or course of infections; vaccinations, including live attenuated measles-mumps-rubella, were given without complications; there was normal growth and development; there were no additional hospitalizations; and the index patient did not have autoimmunity or cancer. EBV plasma load was monitored longitudinally in available samples with quantitative PCR and was detectable at low levels but with an increased frequency compared with those seen in healthy control subjects (positive 4/15 times, see Fig E1, *B*); the index patient was asymptomatic at these occasions. CD4 and CD8 T cells, NK cells, and B cells were sorted from a reactive lymph node cell suspension (derived at age 3 years) and from PBMCs (derived at age 21 years). In both cases EBV was detected in the purified B-cell fraction (but compared with other reports in relatively low amounts: viral loads were 3000 and 2050 viral copies/μg of DNA, respectively[15]).

Family history was notable for 2 childhood deaths (Fig 1, *A*). An older sister died during infancy of an unknown illness. She had otherwise had an uneventful medical history, to our knowledge, although details were limited. An older brother was referred at age 3 years with documented EBV-induced lymphadenopathy, fever, hepatosplenomegaly, and EBV-associated uveitis without evidence of malignancy. He had increasing EBV anti–viral capsid antigen and early antigen titers but absence of Epstein-Barr nuclear antigen seroconversion during follow-up. At initial presentation, the serum IgG level was 10.4 g/L, the IgM level was 2.7 g/L, and the IgA level was 0.4 g/L; IgM levels normalized during a period of 8 months. Lymphocyte phenotyping 8 months after initial presentation showed 0.04 × 10E9/L $CD20^+$ B cells, 0.16 × 10^9/L $CD4^+$ T cells, 2.49 × 10^9/L $CD8^+$ T cells (CD4/CD8 ratio, 0.064), and 1.38 × 10^9/L NK cells. Furthermore, mitogenic and antigenic T-cell responses were absent. After a period of 8 months with severe lymphadenopathy and recurrent episodes of fever, he had aplastic anemia, and 1 month thereafter, he died from fulminant gram-positive bacterial sepsis.

Genomic sequencing

Genomic DNA was isolated from PBMCs or snap-frozen lymph node tissue. Exon-specific M13-tagged primers were used to amplify all coding exons, including flanking regions from the genes *CD27* (NM_001242), *SH2D1A* (NM_002351, encoding SH2 domain protein 1A [SH2D1A] or SLAM-associated protein [SAP]), *XIAP* (NM_001167, encoding X-linked inhibitor of apoptosis [XIAP]), and *PRF1* (NM_001083116, encoding Perforin 1); primer sequences are available on request. PCR products were directly sequenced with M13 sequence primers and BigDye Terminator version 1.1 cycle sequencing kit (Applied Biosystems, Foster City, Calif; www.aplliedbiosystems.com), according to the manufacturer's protocol. Sequencing was performed on a 3130XL genetic analyzer (Applied Biosystems), and sequences were analyzed with SeqScape version 2.5 software (Applied Biosystems).

Flow cytometry

PBMCs were isolated from whole blood by using Ficoll-Hypaque density centrifugation. Antibody staining and fluorescence-activated cell-sorting