DISSEMINATED *CRYPTOSPORIDIUM* INFECTION IN AN INFANT WITH HYPER-IGM SYNDROME CAUSED BY CD40 DEFICIENCY

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We report the case of an infant with severe respiratory infections, chronic diarrhea, failure to thrive, and disseminated *Cryptosporidium parvum* infection. Laboratory investigations disclosed a diagnosis of hyper-IgM syndrome caused by CD40 deficiency. *(J Pediatr 2003;142:194-6)*

The hyper-IgM syndrome (HIGM) is a rare, inherited immune deficiency disorder characterized by recurrent infections associated with low IgG and IgA and normal to increased IgM serum levels.[1] Genetic heterogeneity of HIGM is indicated by the existence of X-linked as well as of autosomal forms of the disease. X-linked hyper-IgM (HIGM1) is caused by defects in the *CD40L/TNFSF5* gene, encoding for CD40 ligand (CD40L), a molecule predominantly expressed by activated CD4+ T-lymphocytes. Loss of interaction between CD40L and its ligand CD40 (constitutively expressed by B-lymphocytes and monocytes) results in impairment of terminal B-lymphocyte differentiation, monocyte activation, and T-lymphocyte priming, thus explaining the severity of clinical features in HIGM1.[2] Revy et al[3] found that defects in the activation-induced cytidine deaminase *(AID)* gene, which is required for immunoglobulin isotype switching and somatic hypermutation, account for an autosomal recessive form of HIGM (HIGM2).[3,4] We reported 3 cases of autosomal recessive HIGM caused by CD40 deficiency (HIGM3).[5]

In this report, we describe the fourth case of HIGM3, in an infant with respiratory distress, growth failure, and disseminated *Cryptosporidium* infection. These data confirm the clinical severity of HIGM3 and suggest that this disease should be considered in the differential diagnosis of combined immunodeficiency.

See editorial, p 99, and related article, p 191.

CASE REPORT

A 12-month-old Turkish girl, born to first-cousin parents, was admitted at Ege University Hospital (Izmir, Turkey) with respiratory distress, hepatomegaly, failure to thrive (body weight, 5350 g; <3rd percentile), and a history of severe respiratory syncytial virus infection. She had a healthy brother and sister, and the family history was negative for infant deaths.

During hospitalization, the infant had necrotizing pneumonia caused by *Pseudomonas aeruginosa,* chronic watery diarrhea, and a severe episode of laryngotracheobronchitis that required tracheostomy. *Cryptosporidium parvum* was repeatedly isolated from the stools and tracheal aspirates, indicating disseminated infection.

Laboratory investigations revealed leukocytosis (20.2-43.7 $\times$ 10^9/L) with eosinophilia (>5 $\times$ 10^9/L), and elevated serum alkaline phosphatase (1858 U/L) and γ-glutamyltranspeptidase (558 U/L). A liver biopsy specimen showed cystic dilation of the bile

CD40L	CD40 ligand
HIGM	Hyper-IgM syndrome
Ig	Immunoglobulin
PCP	*Pneumocystis carinii* pneumonia

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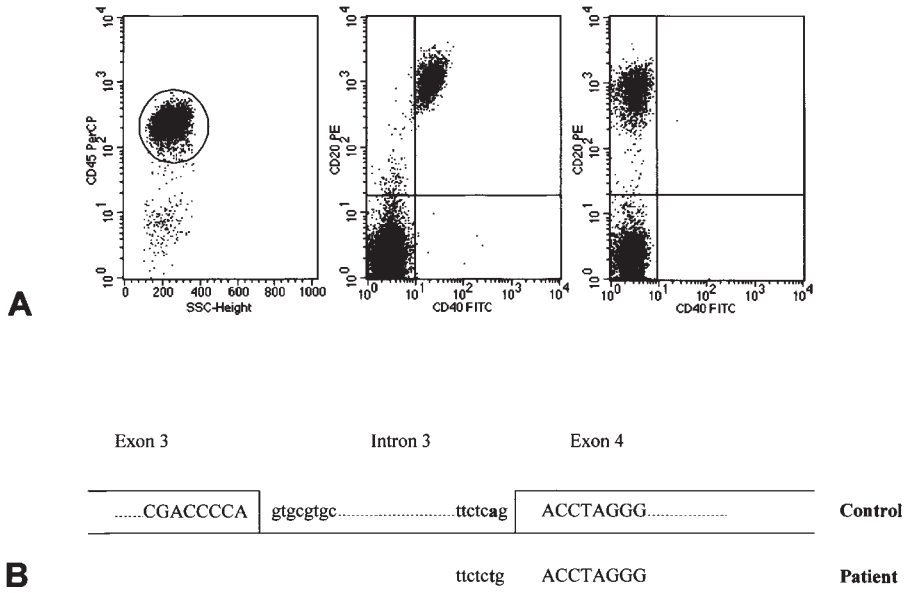

Figure. A, Flow cytometry analysis of CD40 expression by CD45+ lymphoid cells. Peripheral blood mononuclear cells were stained with FITC-conjugated anti-CD40, PE-conjugated anti-CD20, and PerCP-conjugated anti-CD45 monoclonal antibodies and gated on the CD45+ population *(left panel).* Dot plot analysis shows CD20 and CD40 expression in a healthy subject *(middle panel)* and in the patient *(right panel).* Whereas in the former all CD20+ B-lymphocytes are also CD40+, in the latter no CD40 expression is detected on the surface of B-lymphocytes. **B,** Genomic sequencing of the *CD40* gene at the donor and acceptor splice sites of intron 3 in a healthy control subject and in the patient shows that the patient is homozygous for an adenine-to-thymine substitution at position −2 of the acceptor splice site.

ducts and periductal infiltration by eosinophils and polymorphonuclear neutrophils. Computed tomography of the chest showed pulmonary fibrosis, atelectasis, and bronchiectasis. The severity of the clinical course prompted immunologic investigations.

Immunologic and Molecular Investigations

Total neutrophil and lymphocyte counts were normal, as was distribution of peripheral blood lymphocytes (CD_3 56%, CD_{19} 35%, CD_4 24%, CD_8 34%, $CD_{16\text{-}56}$ 11%). Serum immunoglobulin (Ig)G and IgA concentrations were markedly reduced (IgG <146 mg/dL, IgA <5.6 mg/dL), whereas IgM was normal (80 mg/dL). In vitro lymphocyte-proliferative responses to phytohemagglutinin and concanavalin-A were normal. Human immunodeficiency virus testing by enzyme-linked immunosorben assay and Western blot were negative.

The association of severe clinical features with a marked reduction of serum IgG and IgA levels but normal IgM and normal lymphocyte distribution was suggestive of HIGM. HIGM1 was excluded because the patient was a girl. HIGM2 was also unlikely because opportunistic infections are uncommon in this disease. We therefore considered HIGM3 as a possible diagnosis. Flow cytometry analysis showed complete lack of CD40 expression on the surface of circulating B-lymphocytes (Figure, *A*) and of monocytes (data not shown). This prompted us to investigate a possible mutation in the *CD40* gene. Sequencing of all exons and flanking splice sites revealed a novel homozygous mutation (adenine to thymine substitution) at position −2 of the acceptor splice site of intron 3 (Figure, *B*) in a region encoding for the extracellular domain of the protein. Heterozygosity for this mutation was identified in the infant's parents.

DISCUSSION

CD40 is a member of the tumor necrosis factor receptor superfamily and is normally expressed on the surface of B-lymphocytes, mononuclear phagocytes, dendritic cells, endothelial cells, and some activated epithelial cells.[2,6] Activated CD4+ T-lymphocytes express CD40L, which engages CD40 on resting B-lymphocytes and provides a key signal for contact-dependent T-lymphocyte help for B-lymphocyte activation. CD40 activation promotes B-lymphocyte proliferation, Ig isotype switching, generation of memory B-lymphocytes, and germinal center reaction.[7] Ineffective CD40/CD40L interaction is the basis for HIGM1[2] and for the recently described HIGM3.[5]

In keeping with the notion that HIGM1 and HIGM3 result from defects along the same signaling pathway, these disorders also share similar and severe clinical features suggestive of combined immunodeficiency. In particular, infants with HIGM3 are susceptible to *Pneumocystis carinii* pneumonia (PCP),[5] which is also commonly observed in patients with HIGM1.[2,8] We now report disseminated *C parvum* infection and signs of sclerosing cholangitis in a child with HIGM3. Although *C parvum* causes transient diarrhea in persons with normal immunity, it is responsible for protracted, watery diarrhea in patients

with combined immunodeficiency, including HIGM1. In such subjects, *C parvum* often causes biliary tract infection and sclerosing cholangitis.[6,8]

As a whole, these observations indicate that CD40 deficiency (HIGM3) should be considered in the differential diagnosis of infants with clinical signs of combined immunodeficiency. In such patients, routine assays for cell-mediated immunity (lymphocyte count, immunophenotyping of T-lymphocyte subsets, in vitro proliferation to mitogens) may lead to false-negative results. Evaluation of CD40 expression by flow cytometry should be included in the diagnostic workup, particularly if the immunoglobulin profile is suggestive for HIGM.

Optimal treatment of HIGM3 remains a matter of debate. Regular administration of intravenous immune globulin and prophylactic administration of trimethoprim-sulfamethoxazole to prevent PCP are mandatory. Because of the severity of long-term outcome, bone marrow transplantation has been proposed and successfully used in HIGM1.[9] However, the potential benefits of such an approach in HIGM3 are less obvious because it would not correct lack of CD40 expression by endothelial and epithelial cells, which may play a role in the decreased resistance to opportunistic intracellular pathogens in HIGM3.[6,10]

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