Biased Immunoglobulin G (IgG) Subclass Production in a Case of Hyper-IgM Syndrome

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Hyper-immunoglobulin M (IgM) syndrome (HIGM) is a rare heterogeneous primary immune deficiency. We describe a patient with HIGM characterized by skewed production of serum IgG subclasses and normal somatic hypermutation. This case may represent a subgroup of HIGM type 4 that is characterized by a biased switching to the V-region proximal constant regions.

Hyper-immunoglobulin M (IgM) syndrome (HIGM) is typically defined by low serum IgG, IgA, and IgE levels along with raised or normal IgM levels. Mutations of at least five different genes have so far been identified as causing the syndrome, including CD154 (CD40L) (X-linked HIGM1) (1), activation-induced cytidine deaminase (AID) (HIGM2) (11), and CD40 (HIGM3) (5). These three forms of the syndrome are characterized by a complete lack of Ig somatic hypermutation (SHM) and class switch recombination (CSR). Other HIGM conditions are caused by mutations of uracil DNA glycosylase (Ung) and class switch recombination (CSR). Molecular studies have suggested that the defect is downstream of the AID and may involve proteins or AID cofactors that participate in the repair phase of CSR (6). These patients are most susceptible to recurrent bacterial infections, consistent with a lack of production of the IgG2 subclass (6).

Clinical findings. We describe a 15-year-old female with autoimmune hypothyroidism that presented with an 18-month history of increasing dyspnea and recurrent pneumonia unresponsive to antibiotics. Findings on physical examination were a large thyroid and very enlarged tonsils. A lung biopsy showed lymphoid interstitial pneumonitis with areas of fibrosis and bronchiolitis obliterans, although histopathology, culture, or molecular studies identified no pathogens. Past medical history was significant for recurrent otitis media from infancy and persistent axillary adenopathy and splenomegaly from age 3 years. At age 4, a lymph node biopsy had shown follicular hyperplasia and germinal centers of variable size and shape. Laboratory analysis revealed normal serum IgM (patient, 0.78 g/liter; normal, 0.5 to 1.7 g/liter) with low IgG (patient, 0.62 g/liter; normal, 5.49 to 15.84 g/liter), absent IgA (patient, <0.07 g/liter; normal, 0.61 to 3.48 g/liter), and absent IgE (patient, <2 μg/liter; normal, 32 to 98 μg/liter), indicating an Ig isotype switching defect. Closer inspection of serum IgG isotypes revealed that IgG2 and IgG4 were absent (<0.02 g/liter), IgG1 was markedly reduced (patient, 0.28 g/liter; normal, 4 to 7 g/liter), whereas IgG3 was just below normal range (patient, 0.29 g/liter; normal, 0.45 to 0.7 g/liter). Thus, the low serum IgG was biased to a 50:50 ratio of IgG1 and IgG3 rather than the normal distribution of predominantly IgG1 (66%) and IgG2 (22%), with minor proportions of IgG3 and IgG4. Further serology showed absent isohemagglutinins and absence of memory antibodies to measles, mumps, and rubella (after two doses of each vaccine), varicella-zoster (postinfection), and tetanus (after six doses of vaccine). Diphtheria antibodies were low but detectable. B- and T-lymphocyte numbers were normal. B-lymphocyte CD19 and CD27 expression were normal, as was T-lymphocyte proliferation stimulated by phytohemagglutinin and pokeweed mitogen. In vitro antigen-specific lymphocyte proliferation was present for rubella, mumps, measles, varicella, and candida. Given her compromised lung condition, with a potential poor prognosis, she was immediately started on regular intravenous Ig therapy, which obviated further study of in vivo antibody responses, such as the responses to previously administered vaccines, neonatogens, or polysaccharide antigens.

Molecular investigations. Normal patterns of X-chromosome inactivation and CD40L expression and normal B-lymphocyte CD40 expression allowed us to exclude the diagnosis of HIGM types 1 and 3. Expression of AID mRNA in peripheral blood B lymphocytes stimulated with interleukin-4 or CD40 ligation was normal. The sequence of AID mRNA and genomic DNA exons were also normal, eliminating the possibility of HIGM2 syndrome. To analyze the SHM status and determine HIGM4 or Ung deficiency, we analyzed IgM transcripts (\(V_{H}-C_{\mu}\)) amplified by reverse transcription-PCR from CD27+ memory B lymphocytes. Ninety-five percent (19/20) of clones displayed evidence of somatic mutations and in total, 165 mutations were identified from 5,855 total bases sequenced (Fig. 1). This corresponds to a mutation frequency of 2.8% (normal range, 2.6 to 6.3%) and is very similar to the mean mutation frequency of 3.3% previously found with
HIGM4 patients (6). Furthermore, the pattern of mutated bases reflects the normal distribution of somatic mutations with transitions at G/C base pairs favored (12). We found very little evidence of SHM accumulation in IgM transcripts isolated from naive CD27- B lymphocytes, consistent with their lack of antigenic stimulation (Fig. 1).

Taken together, these results indicate that this patient displays a clinical and laboratory phenotype consistent with the diagnosis of HIGM4. Clinically she had recurrent infections, lymphoid hyperplasia, and autoimmune thyroiditis. Detailed molecular studies excluded the diagnosis of HIGM types 1 to 3. We were intrigued by the biased IgG subclass production and the potential of this feature in further delineation of individuals with CSR defects. Strikingly, although this patient's serum IgG was markedly reduced to less than 10% of normal levels, levels of IgG3 were almost normal. Thus, there was a redistribution dominated by IgG1 followed by IgG2, with minor levels of IgG3 and IgG4.

The most comprehensive study of HIGM4 to date included 15 patients, of which approximately half (8/15) displayed residual serum IgG levels greater than 2.0 g/liter, and interestingly pooled analysis of IgG subclasses of four patients displayed a similar pattern as we describe for the single patient herein (6). Interestingly, several immunodeficient patients with hypogammaglobulinemia but with relative preservation of serum IgG3 levels have been described (2, 4, 8). It is unknown whether these previously diagnosed patients could be classified as HIGM4, since no analysis of SHM status and spectrum was performed. To our knowledge then, this is the first patient diagnosed with HIGM4 syndrome and severe hypogammaglobulinemia in which IgG subclass imbalance has been described. We speculate that the relative increase in serum levels of IgG1 and IgG3 over IgA, IgG2, and IgG4 may be related to the respective proximity of these constant region genes to the rearranged variable gene within the Ig heavy-chain locus.

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