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

G. R. McLean,1* K. K. Miller,2 J. W. Schrader,1 and A. K. Junker2*

The Biomedical Research Centre, University of British Columbia,1 and Department of Pediatrics, British Columbia Children’s Hospital,2 Vancouver, Canada

Received 26 July 2004/Returned for modification 20 August 2004/Accepted 9 September 2004

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) (HIGM4) (3, 6) or associated with ectodermal dysplasia due to mutations in the X-linked nuclear factor (7) or associated with ectodermal dysplasia due to mutations in class switch recombination (CSR). Other HIGM conditions are caused by mutations of uracil DNA glycosylase (Ung) (7) or associated with ectodermal dysplasia due to mutations in the X-linked nuclear factor κB essential modulator (NEMO) (9).

To date, 48 patients with HIGM4 (3, 6) or unknown HIGM (10) have been described in the literature. The genetic defect(s), yet undetermined, seems likely to be associated with the process of CSR in that there is normal SHM but impaired 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, neoantigens, 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 (VH1-3-Cμ) 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

* Corresponding author. Mailing address for G. R. McLean: Division of Infectious & Immunological Diseases, BC Children’s Hospital, Room K4-223, 4480 Oak St., Vancouver, BC, Canada V5H 3V4. Phone: (604) 875-3591. Fax: (604) 875-2414. E-mail: ajunker@cw.bc.ca.
the respective proximity of these constant region genes to the
of IgG1 and IgG3 over IgA, IgG2, and IgG4 may be related to
described. We speculate that the relative increase in serum levels
globulinemia in which IgG subclass imbalance has been de-
diagnosed with HIGM4 syndrome and severe hypogamma-
performed. To our knowledge then, this is the first patient
whether these previously diagnosed patients could be classified
rum IgG3 levels have been described (2, 4, 8). It is unknown
hypogammaglobulinemia but with relative preservation of se-
played a similar pattern as we describe for the single patient
molecular studies excluded the diagnosis of HIGM types 1 to
lymphoid hyperplasia, and autoimmune thyroiditis. Detailed
diagnosis of HIGM4. Clinically she had recurrent infections,
lack of antigenic stimulation (Fig. 1).

Taken together, these results indicate that this patient dis-
plays 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 indi-
viduals with CSR defects. Strikingly, although this patient's
serum IgG was markedly reduced to less than 10% of normal
values, levels of IgG3 were almost normal. Thus, there was a
preponderance of IgG3 and IgG1, in contrast to the normal
distribution 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 re-
idual serum IgG levels greater than 2.0 g/liter, and interest-
ingly pooled analysis of IgG subclasses of four patients dis-
played 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 hypogamma-
oglobulinemia in which IgG subclass imbalance has been de-
scribed. 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.

FIG. 1. Somatic hypermutation analysis of V 
transcripts from HIGM patient B cells. Total RNA was prepared from flow cytometry-sorted
B cells, mRNA was reverse transcribed, and V transcripts were amplified with Pfu polymerase using primers V 3F (TGCGCTACCAAC
ATGGAAGTTCGCGGCTG) and µR (CTCAGAAGCTTAGCAGCTCACGCAATC). The data are presented as the number of specific nucleotide
mutations compared to the nearest germ line V element. (A) CD27 - B cells. A total of nine clones were sequenced and 17 mutations were
identified from 2,785 total nucleotides (mutation frequency = 0.6%). (B) CD27 + B cells. A total of 20 clones were sequenced and 165 mutations
were identified from 5,855 total nucleotides (mutation frequency = 2.8%). Targeting at G/C was 61% (normal, 62 to 66%), and 59% (normal, 57
to 63%) of mutations were transitions.

REFERENCES
1. Allen, R. C., R. J. Armitage, M. E. Conley, H. Rosenblatt, N. A. Jenkins,
N. G. Copeland, M. A. Bedell, S. Edelhoff, C. M. Distech, D. K. Simoneneaux,
Griscelli, and J. L. Preud’Homme. 1989. Serum IgG subclass levels in pa-
ients with primary immunodeficiency syndromes or abnormal susceptibility
noglobulin G transcripts in a case of hyper-immunoglobulin M syndrome
similar to type 4. Immunology 111:212–222.
Calvert. 1990. B cell differentiation and lymphocyte surface phenotype in
late onset hyperimmunoglobulinemia. Dis. Markers 869–89.
5. Ferrari, S., S. Gilliani, A. Insalaco, A. Al-Ghonaium, A. R. Soresina, M.
Loubser, M. A. Avanzini, M. Marconi, R. Badalato, A. G. Ugazio, Y. Levy,
Mutations of CD40 gene cause an autosomal recessive form of immunode-
Nagendran, P. Wood, C. Glastré, F. Sarrot-Reynaud, O. Hermine, M. For-
veille, P. Revy, A. Fischer, and A. Durandy. 2003. Hyper-IgM syndrome type
4 with a B lymphocyte-intrinsic selective deficiency in Ig class-switch recom-
7. Imai, K., G. Shupphasang, W. I. Lee, P. Revy, S. Nonoyama, N. Catalan, L. Vel,
M. Forveille, B. Kavli, H. E. Krokan, H. D. Ochs, A. Fischer, and A. Du-
randy. 2003. Human uracil-DNA glycosylase deficiency associated with pro-
foundly impaired immunoglobulin class-switch recombination. Nat. Immu-
nol. 4:1023–1028.
1989. Development of hypogammaglobulinemia in a patient with common
Specific missense mutations in NEMO result in hyper-IgM syndrome with
2003. Inducible CO-stimulator molecule, a candidate gene for defective
isotype switching, is normal in patients with hyper-IgM syndrome of un-
M. Forveille, R. Dufourcq-Labelouse, A. Gennery, I. Tezcan, F. Erosh, H.
Kayserili, A. G. Ugazio, N. Brousse, M. Muramatsu, L. D. Notarangelo, K.
cytidine deaminase (AID) deficiency causes the autosomal recessive form of
the hyper-IgM syndrome (HIGM2). Cell 102:565–575.
12. Wagner, S. D., and M. S. Neuberger. 1996. Somatic hypermutation of