Molecular basis of selective IgG2 deficiency. The mutated membrane-bound form of gamma2 heavy chain caused complete IGG2 deficiency in two Japanese siblings.

H Tashita, … , K Kasahara, N Kondo


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Molecular Basis of Selective IgG2 Deficiency

The Mutated Membrane-bound Form of γ2 Heavy Chain Caused Complete IgG2 Deficiency in Two Japanese Siblings

Hideaki Tashita, Toshiyuki Fukao, Hideo Kaneko, Takahide Teramoto, Ryosuke Inoue, Kimiko Kasahara, and Naomi Kondo

Department of Pediatrics, Gifu University School of Medicine, Gifu, Gifu 500-8076, Japan

Abstract

Patients with IgG2 deficiency have recurrent sinopulmonary infections caused by Pneumococcus and Hemophilus. Hereditary and selective IgG2 deficiency was suspected in two Japanese siblings whose serum IgG2 levels were under detection limits, while other serum levels of immunoglobulin subclasses were within normal ranges. Expression level of spontaneous germline Cγ2 transcript was normal, but that of the spontaneous mature Cγ2 transcript was greatly decreased in the patients’ PBMCs, suggesting the presence of a defect at or after the class switch to Cγ2. We sequenced the Cγ2 gene region, and in both patients a homozygous one-base insertion (1793insG) was present in exon 4 of the Cγ2 gene, just upstream from the alternative splice site for M exons. The mutant membrane-bound γ2 heavy chain loses the transmembrane domain and the evolutionarily conserved cytoplasmic domain. Considering several lines of evidence showing that intact expression of the membrane-bound heavy chain is essential for a normal response of B cells and production of secreted immunoglobulin in mice, we concluded that 1793insG is responsible for selective and complete IgG2 deficiency in these two siblings. This is the first documentation of a mutation in human selective IgG2 deficiency. (J. Clin Invest. 1998. 101:677–681.) Key words: immunodeficiency • mutation • alternative splicing • M exon • IgG subclass

Introduction

The B cell antigen receptor (BCR) initially expressed on B cells consists of membrane-bound immunoglobulin made up of μ, heavy (H) chains plus light chains associated with the Igα-β heterodimer (1). When B cells are stimulated with antigen, they often undergo isotype switching leading to expression of H chains of other classes. These other H chains differ from those of μ and δ in that they possess cytoplasmic tails of 28 (γ, ε) and 16 (α) amino acids, which have been highly conserved throughout evolution. Membrane-bound immunoglobulin expression is essential for generation of efficient primary and secondary immunoglobulin responses. The primary immunoglobulin response as well as the expansion, maintenance, or both, of immunoglobulin-bearing memory B cells, depends strongly on the cytoplasmic tail of the heavy chain in mice (3–5).

IgG subclass deficiency is an immunodeficiency associated with the absence or severe reduction in the level of one or two subclass(es), but with normal or increased levels of other subclass(es). Only a homozygous deletion of the Ig H chain constant region genes has been found in some patients with IgG subclass deficiencies (6). The molecular basis of selective IgG2 deficiency has not been defined. We investigated molecular events of selective and complete IgG2 deficiency in two Japanese siblings in whom serum IgG2 levels were retained under the detection limit for at least 4 yr; some transient IgG2-deficient children show normalization without therapy (7). We identified a homozygous 1793insG mutation in Cγ2 exon 4, which resulted in loss of the transmembrane domain and the evolutionarily conserved cytoplasmic domain in a membrane-bound γ2 heavy chain (mγ2HC).

Methods

Case presentation. The proband (5-yr-old boy) and his elder brother (10 yr old), both Japanese, were examined. The parents were not consanguineous. Otitis media and respiratory infection such as pneumonia had occurred repeatedly in both patients. Serum IgG2 was undetectable (< 2.7 mg/dl), and the levels of other immunoglobulins, including other IgG subclasses, were practically normal in both patients. The specific IgG2 antibody to Streptococcus pneumoniae was also undetectable. The IgG2 level in the mother was within a normal range. Samples from the father were not available.

Serum IgG subclass level. The serum IgG subclass levels were measured, using ELISA as described (8). We used highly purified monoclonal antibodies against each of the four human IgG subclasses (HP6012 for IgG1, HP6014 for IgG2, HP6050 for IgG3, and HP6011 for IgG4, as recommended by the World Health Organization).

RNA preparation and reverse transcription. PBMCs were isolated from heparinized blood by gradient centrifugation in Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden). Total cellular RNA was extracted from the 1.0 × 10⁹ PBMCs using Isogen (Nippon Gene, Tokyo, Japan). Total RNA was reverse transcribed at 37°C for 60 min with 200 U of MMLV reverse transcriptase (GIBCO-BRL, Gaithersburg, MD), and 30 ng of oligo d(T) primer in 20 μl of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.5 mM dNTPs, and 20 U of RNase inhibitor (Pharmacia Fine Chemicals).

Preparation of a Sty I fragment for competitor DNA. PCR amplification of interleukin-12 receptor CDNA (9) was carried out with flanking primers and cycling conditions as follows: forward, 5'-1tqcaaggcggagagacct-3`; reverse, 5'-12hoTCCCTGCCCAATTGCC-
Table I. Oligonucleotide Primers

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<tr>
<th>Name</th>
<th>Direction</th>
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<th>Position</th>
<th>Target</th>
<th>Reference</th>
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</thead>
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<td>Iγ 2</td>
<td>germline Cγ 2 transcript</td>
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<td>HA1</td>
<td>antisense</td>
<td>922ACTCGACACAACATTTGCC</td>
<td>Cy 2 exon2 (hinge)</td>
<td>germline and mature Cγ 2 transcripts</td>
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<tr>
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<td>mature Cγ 2 transcript</td>
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<td>HS</td>
<td>sense</td>
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<td>Cy 2 exon 2 (hinge)</td>
<td>Cγ 2 fragment (for Sma I digestion)</td>
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</tr>
<tr>
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<td>antisense</td>
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<td>γ 2 M exons</td>
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<td>11</td>
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<tr>
<td>CA</td>
<td>antisense</td>
<td>tcagattcagctggctgggccac</td>
<td>downstream from Cy 2 exon4</td>
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<td>sense</td>
<td>193ccctccgtagctgttagcttctggcttt</td>
<td>downstream from Cy 2 exon4</td>
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<td>sense</td>
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<td>Iγ 2</td>
<td>Iγ 2-Cγ 2 fragment (first PCR)</td>
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<td>sense</td>
<td>139ggcaggagctttgaggctttcag</td>
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<td>inverted repeat of 5' Sy 2 segment</td>
<td>Sy 2 (second PCR)</td>
<td>10</td>
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Oligonucleotide sequences for γ 2-specific amplification. de novo Eco RI site was introduced CS1 and CA primers for cloning (underlined). *n = A, G, C, or T.

CCAGA-3'; 95°C for 1 min, 60°C for 1 min 30 s, and 72°C for 3 min for 35 cycles. The product is 1284 bp in length and yields the expected 113 bp, 191 bp, 233 bp, 348 bp, and 399 bp fragments after Sty I digestion. The 233-bp fragment was separated by polyacrylamide-gel electrophoresis, and purified.

Oligomers used in following experiments. Fig. 1 schematically shows the locations of oligomers in the regions of JH, Iγ2, Sy2, Cy2, and M exons. Table I shows sequences of oligomers and some information. The nucleotide numbers on oligomers are given in the reference. Capital letters were used in cases of coding sequences, and small letters were used in cases of noncoding sequences.

Quantitative PCR for germline Cγ 2 transcript. PCR amplification of germline Cγ 2 transcript was carried out with IS1 and HA1 primers and cycling conditions as follows: 94°C for 45 s, 65°C for 1 min, and 72°C for 1 min 30 s for 35 cycles. The plasmid containing 341 bp cDNA from germline Cγ 2 transcript was partially substituted with the 233-bp Sty I fragment from interleukin-12 receptor cDNA, and was used as a competitor DNA. The PCR product of the wild-type was 341 bp, and that of the competitor was 287 bp. Each template contained the same amounts of cDNA from RNA extracted from 7.3 × 10^6 PBMCs, and one of several tenfold dilutions of germline Cγ 2 transcript competitor from 1.0 × 10^{-1} to 1.0 × 10^{-3} attomole. The equivalent point was determined by polyaerylamide gel electrophoresis.

Quantitative PCR for mature Cγ 2 transcript. To amplify the mature Cγ 2 transcript specifically, we prepared a common 5' primer for JH1, JH4, and JHS genes, JHS (12). PCR amplification of the mature Cγ 2 transcript was carried out with JHS and HA1 primers, and cycling conditions were as follows: 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min for 35 cycles. The competitor DNA for mature Cγ 2 transcript was generated as follows: the plasmid containing 335-bp cDNA from the mature Cγ 2 transcript was partially substituted with the 233-bp Sty I fragment from interleukin-12 receptor cDNA, and was used as a competitor DNA. The PCR product of the wild-type is 335 bp, and that of the competitor is 301 bp. Each template contained the same amounts of cDNA from RNA extracted from 7.3 × 10^6 PBMCs and one of several tenfold dilutions of mature Cγ 2 transcript competitor from 1.0 × 10^{-1} to 1.0 × 10^{-3} attomole. The equivalent point was determined by polyaerylamide gel electrophoresis.

Amplification of the genomic Sy2 region. Genomic DNA was purified from a polynuclear cell fraction with a Sepa Gene (Sanko Jyunyaku, Tokyo, Japan) according to the manufacturer's instruction. Nested PCR amplification of genomic region for Sy2 was carried out with IS2 and HA2 primers (first PCR), SS and SA primers (second PCR), and cycling conditions as follows: 94°C for 1 min, 65°C for 2 min, 72°C for 6 min for 40 cycles (first PCR), 94°C for 1 min, 65°C for 2 min, and 72°C for 4 min for 40 cycles (second PCR).

Amplification of the genomic Cγ 2 region. PCR amplification of genomic region for Cy2 was carried out with CS1 and CA primers and cycling conditions as follows: 94°C for 1 min, 60°C for 1 min, 72°C for 5 min for 5 cycles; and 94°C for 1 min, 60°C for 2 min, and 72°C for 2 min for 40 cycles.

Cloning of M exons for my2HC. Human Cγ 2 gene cloned by a Charon 4A phage was kindly donated by Dr. T. Honjo (Kyoto University; 13). We subcloned a 6.4-kb Hind III fragment including Cγ 2 exons to pTZ18U (United States Biochemical Corp., Cleveland, OH) from the phage clone. We prepared the primers on exon 4 of Cγ 2 and the highly conserved sequence of human γ M2 exons (MA; 14, 15). Cycle conditions were as follows: 94°C for 1 min, 65°C for 2 min, and 72°C for 5 min for 40 cycles. The fragment was cloned into pT7Blue T-vector (Novagen, Inc., Madison, WI) and analyzed.

Amplification of cDNA for my2HC. Complementary DNA for my2HC was amplified with HS and MA primers and cycling conditions as follows: 94°C for 1 min, 65°C for 2 min, and 72°C for 5 min for 40 cycles.

Results

Quantitative reverse transcription (RT)-PCR for germline Cγ 2 transcript. Expression of germline transcripts of each constant H region, except for C8, is apparently essential for class switch recombination (16, 17). The germline Cγ 2 transcript was reported to be expressed spontaneously in PBMCs (18). We prepared 5' Iγ2 and 3' Cγ 2 specific primers (Fig. 1), and quantitative RT-PCR for germline Cγ 2 transcript was carried out. The relative quantities of germline Cγ 2 transcript were assayed by the equivalent points of competitive PCR in the polyaerylamide-gel electrophoresis patterns (Fig. 2A). Proband's target cDNAs and competitor were almost equivalent at 3.3 × 10^{-2} attomole, and those of a control were also equivalent at 3.3 × 10^{-2} attomole. As shown in Fig. 2 C, the relative quantity of germline Cγ 2 transcript, determined at the equivalent points
in this competitive PCR, showed no apparent difference between patients and controls.

Quantitative RT-PCR for mature Cγ2 transcript. Since spontaneous IgG2 production in PBMCs can occur (19), spontaneous expression of mature Cγ2 transcript can also be expected. To amplify the mature Cγ2 transcript specifically, we set a sense primer on the conserved sequence for JH1, JH4, and JH5 genes (12), and an antisense primer on the hinge region of sense primer on the conserved sequence for JH1, JH4, and JH5 genes (12), and an antisense primer on the hinge region of

Amplification and sequencing of genomic Cγ2 region. Because of the high degree of homology between the human Cγ1, Cγ2, Cγ3, and Cγ4 genes (the percentage of homology in the coding regions is 95.3–97.9%, with a homology of 93.3–95.7% in noncoding areas; 20), we designed a pair of primers that would specifically amplify the Cγ2 gene (Fig. 1). The product of the proband’s Cγ2 region, which includes exons 1–4 for the Cγ2 gene, was entirely sequenced.

When the nucleotide is numbered according to the database EMBL J00230, the termination codon of the secreted form of γ2 H chain (sγ2HC) is 1020TGA. We identified one-base replacement from T to C (1790T/C), and one-base insertion of G between nucleotide numbers 1792 and 1793 (1792insG) in the proband (Fig. 3 A). Sequence analysis revealed that both 1790T/C and 1792insG were detected homozygously in both the patients’ DNAs, heterozygously in their mother’s DNA, but not detected in 28 healthy controls. These two mutations, which had only a 3-bp distance from each other, were linked in the alleles tested. Therefore, using 1790T/C, which creates a new Sma I site, we sequenced genomic DNAs of 65 independent normal controls (130 alleles), but detected no 1790T/C mutant allele (Fig. 3 B). 1790T/C causes no amino acid change, and 1792insG causes only a deletion of the last lysine residue in sγ2HC by a frame shift (Fig. 4 A).

Cloning and sequencing M exons for my2HC. To assess the effects of these mutations on my2HC, we cloned and sequenced the two M exons for my2HC. Sequences of γ2 M
exons (DDBJ, EMBL, and GenBank accession numbers AB006775 and AB006776) were highly homologous to those of other \( \gamma \) M exons (14, 15). The \( \gamma 2 \) M1 exon was identified \( \sim 1.1 \) kbp downstream from the termination codon for \( \gamma 2 \) HC. The M2 exon started 447 bp downstream from the last base of the M1 exon.

**Amplification of cDNA for \( \gamma 2 \) HC.** Thereafter, we designed a pair of primers so that the cDNA for \( \gamma 2 \) HC was specifically amplified (Fig. 1). The cDNAs were successfully amplified in controls and in both patients. The alternative splice site was found at \( 1793 \text{G/GT} \) in the CY2 exon 4 just downstream from the mutation site (Fig. 4A). Hence, 1793insG resulted in a frame shift of mRNA for \( \gamma 2 \) HC, causing complete changes of amino acid sequence encoded by M1 and M2 exons and generated an abnormal 117 amino acid sequence (Fig. 4, A and B). The cDNAs for \( \gamma 2 \) HC were then subjected to Sma I restriction assay. As expected, the mutations were homozygous in both patients’ cDNAs, but the mutations were nil in 20 healthy controls’ cDNAs (Fig. 3 C).

**Discussion**

We cloned M1 and M2 exons for \( \gamma 2 \) HC, identified the alternative splice site in CY2 exon 4 for M exons, and showed that 1793insG in CY2 exon 4 caused a frame shift in \( \gamma 2 \) HC that resulted in complete changes of amino acid sequence encoded by M1 and M2 exons. We propose that 1793insG is the cause of complete and selective IgG2 deficiency in these two Japanese siblings. Since the heterozygous mother had a normal IgG2 level, this mutation is unlikely to account for the large number of individuals with a low IgG2 level.

There are at least two possibilities that would explain the mechanism of IgG2 deficiency with the frame shift of \( \gamma 2 \) HC. One is that this mutation results in complete loss of function and structure as a BCR, and the mutant \( \gamma 2 \) HC never appears on the B cell surface. The other is that the mutant \( \gamma 2 \) HC, which lost the conserved motif in the cytoplasmic tail, could be expressed on the B cell surface; however, it could not complete the signal transduction or antigen processing. It was conceivable that the first case was the counterpart of mice lacking the transmembrane and cytoplasmic domains of \( \gamma 1 \) HC or \( \gamma 2 \) HC, and the latter case was mice lacking most of the cytoplasmic tail of \( \gamma 1 \) HC or \( \gamma 2 \) HC, respectively (3, 4). The levels of serum IgG1 and IgE were reduced to a lesser extent in mice lacking the transmembrane domains than in mice lacking partial cytoplasmic domains. Because of a complete deficiency of serum IgG2, it seems likely that the patient’s B cells could not express \( \gamma 2 \) HC on the surface. Hydropathy profile of normal and mutant sequences encoded by M exons was investigated according to Kyte and Doolittle (21). The average hydropathy of 19-residue segments of normal \( \gamma 2 \) HC transmembrane domain was \( +2 \), satisfying a condition of membrane-spanning sequences. On the other hand, most of the mutant sequence encoded by M exons was hydrophilic, and the average hydropathy of any 19-residue segment of the mutant sequence was \( < +1.2 \), which strongly suggests that there is no membrane-spanning sequence in the mutant sequence (data not shown).

Weiser et al. reported that transformed B cells expressing an IgG2a BCR require the cytoplasmic tail of \( \gamma 2 a \), and specifically a tyrosine-based motif in this structure (5) that is also present in human \( \gamma 2 \) HC for efficient presentation of antigen to T cells after surface immunoglobulin-mediated internalization. The frame shift mutation lost the tyrosine-based motif (5). Based on these results in mice, we conclude that the frame shift of \( \gamma 2 \) HC caused by 1793insG resulted in a complete and selective IgG2 subclass deficiency.

The above cases including our case were those of a selective immunoglobulin deficiency. Cases of defects in the \( \mu \) H chain were also noted both in mice and humans. In a model mouse with a disrupted M exon of the \( \mu \) H chain gene, a complete absence of B cell production and profound hypogammaglobulinemia were evident (22). Mutations in the \( \mu \) H chain gene were also identified in patients with agammaglobulinemia who did not have mutations in Bruton’s tyrosine kinase (23). These observations mean that an intact membrane-bound \( \mu \) H chain, which mediates signals into pro-B cells via...
BCR, is essential for B cell development in both mice and humans. The efficient primary and secondary immunoglobulin responses depend on the appropriate expression of the membrane-bound form of the H chain in humans as well as mice.

Acknowledgments

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