A novel splice variant of FcγRIIa: A risk factor for anaphylaxis in patients with hypogammaglobulinemia

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Background: Our index case was a patient with common variable immunodeficiency (CVID). She had anaphylactoid reactions on administration of intravenous immunoglobulin (IVIg) associated with the presence of IgG antibodies against IgA.
Objective: We sought to determine the role of Fcγ receptor (FcγR) IIa in IVIg-induced anaphylactoid reactions.
Methods: Neutrophils and PBMCs were isolated from healthy subjects and IVIg-treated patients. FcγRIIa mRNA and DNA were analyzed by using real-time PCR and sequencing. IgG-mediated elastase release and intracellular Ca^{2+} mobilization were determined in neutrophils and transfected cell lines, respectively.
Results: A novel splice variant of FcγRIIa containing an expressed cryptic exon 6* (FcγRIIa^{exon6*}) was identified in our index patient. This exon is normally spliced out of all FcγRII isoforms, except the inhibitory FcγRIIb1. Compared with healthy control subjects, the heterozygous *FCGR2A*$^{c.742+871A>G}$ mutation was more frequent in patients with CVID (n = 53, $P < .013$). Expression in patients with CVID was associated with anaphylaxis on IVIg infusion ($P = .002$). On screening of additional IVIg-treated patient cohorts, we identified 6 *FCGR2A*$^{c.742+871A>G}$ allele–positive patients with Kawasaki disease (n = 208) and 1 patient with idiopathic thrombocytopenia (n = 93). None had adverse reactions to IVIg. Moreover, FcγRIIa^{exon6*} was also demonstrated in asymptomatic family members. Functional studies in primary cells and transfected murine cells demonstrated enhanced cellular activation by FcγRIIa^{exon6*} compared with its native form, as shown by increased elastase release and intracellular calcium mobilization.
Conclusion: A novel splice variant, FcγRIIa^{exon6*}, was characterized as a low-frequency allele, coding for a gain-of-function receptor for IgG. In the presence of immune complexes, FcγRIIa^{exon6*} can contribute to anaphylaxis in patients with CVID. (J Allergy Clin Immunol 2013;131:1408-16.)

Key words: *Anaphylaxis, common variable immunodeficiency, idiopathic thrombocytopenia, intravenous immunoglobulin, Fcγ receptors, Kawasaki disease, splice variant, neutrophils, elastase*

Human Fcγ receptors (FcγRs) are cell-surface glycoproteins that bind the Fc region of IgG. FcγRs are encoded on chromosome 1q23.[1,2] Three types of FcγRs, types I, II, and III, are discriminated based on their affinity for monomeric IgG. Type I is a high-affinity receptor, whereas types II and III are low-affinity receptors.[3] Of these receptors, FcγRII is the most widely distributed because it is expressed on most types of blood cells.[4] Deficiencies of FcγRII have never been observed, and it is assumed that the lack of FcγRII will be lethal. FcγRII is the only FcγR that contains its own signaling motif, whereas FcγRI and FcγRIII are dependent on association with another molecule for signal transduction.

Several isoforms of FcγRII exist, which are highly homologous in their extracellular and transmembrane regions but differ in their intracellular domains.[5,6] In this domain FcγRII contains, depending on the isoform, either an immunoreceptor tyrosine-based activation motif or an immunoreceptor tyrosine-based inhibitory motif. FcγRIIa and FcγRIIc contain an immunoreceptor tyrosine-based activation motif and therefore function as activating receptors, whereas FcγRIIb contains an immunoreceptor tyrosine-based inhibitory motif and acts as an inhibitory receptor.[7-9] The isoform FcγRIIb has 2 functional splice variants: FcγRIIb1 and FcγRIIb2. Apart from differential expression patterns, these splice variants differ in the presence or absence of exon 6, respectively.[10] Similar to FcγRIIb2, FcγRIIa and FcγRIIc lack this exon (although it is not recognized as a true exon in these isoforms), whereas FcγRIIb1 does contain exon 6. To date, splice variants of FcγRIIa have not been reported.

Because rodents lack an FcγRIIa homolog, transgenic animals have been generated to study (human) FcγRIIa *in vivo*.[11] In comparison with their wild-type littermates, induction of thrombocytopenia by antibodies was more severe in hFcγIIa transgenic mice,[11] suggesting an important proinflammatory role for FcγRIIa in disease. In the same mouse model, FcγRIIa has recently been shown to be sufficient to trigger both anaphylaxis and airway inflammation in response to immune complexes.[12] Other experimental animal models and *in vitro* studies suggest that the ratio between activating and inhibitory FcγRs determines the responsiveness of immune cells to immune complexes.[13,14]

We have developed a highly sensitive real-time PCR for the quantification of mRNA of FcγRII isoforms[15] to test whether mRNA levels of FcγRII in neutrophils of healthy volunteers and various patient cohorts correlate with the cellular

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Abbreviations used
$[Ca^{2+}]_i$: Intracellular Ca^{2+} ions
CVID: Common variable immunodeficiency
FcγR: Fcγ receptor
FITC: Fluorescein isothiocyanate
fMLP: N-formyl-methionyl-leucyl-phenylalanine
IMIg: Intramuscular immunoglobulin
ITP: Idiopathic thrombocytopenia
IVIg: Intravenous immunoglobulin
KD: Kawasaki disease
SNP: Single nucleotide polymorphism

responsiveness to IgG and immune complexes. For this reason, we investigated the FcγRII mRNA levels in patients whose primary treatment is intravenous immunoglobulin (IVIg) as substitution or immunomodulation therapy: patients with hypogammaglobulinemia, idiopathic thrombocytopenia (ITP), and Kawasaki disease (KD). ITP is a common disease entity. In contrast, KD is a rare febrile childhood vasculitis of medium-sized arteries of unknown cause. This last disease is diagnosed according to a set of clinical criteria.[16] In 20% to 25% patients with KD, coronary artery aneurysms develop, which renders KD the leading cause of acquired heart disease in subjects less than 5 years of age. The standard treatment for KD consists of a single high dose of IVIg (2 g/kg) in combination with oral aspirin to reduce the risk and severity of coronary artery aneurysms.[17]

We identified aberrant FcγRIIa in some of these patients. The index patient had an acquired form of hypogammaglobulinemia, common variable immunodeficiency (CVID), and needed antibody supplementation therapy to combat recurrent infections. She had a severe adverse reaction to her first dose of IVIg. The presence of aberrant FcγRIIa was further studied in a larger cohort of patients with CVID with similar clinical findings of anaphylaxis. Although patients with aberrant FcγRIIa were also recognized among patients with ITP and those with KD, clinical anaphylaxis did not occur in these patients on IVIg treatment.

Here we report a novel gain-of-function FcγRIIa splice variant containing a cryptic exon 6*, FcγRIIa^{exon6*}, which was expressed in granulocytes and monocytes and might be present in the healthy population as a low-frequency mutation associated with CVID and anaphylactoid reactions toward IVIg infusion.

METHODS

Patient description

A 28-year-old white woman was given a diagnosis of CVID at the age of 26 years when, after a period of recurrent upper airway infections and chronic *Giardia lamblia*–related diarrhea, examination revealed widespread gastric and intestinal nodular lymphoid hyperplasia in combination with low serum immunoglobulin levels. There was no history of infectious or autoimmune diseases among her family members.

After the start of the first IVIg infusion, the patient complained of abdominal pain and nausea while having a generalized rash, a respiration rate of 36 breaths/min, and tachycardia (136 beats/min), with a decrease in blood pressure from 137/95 to 106/74 mm Hg. Half an hour later, she experienced chills followed by fever (39.2°C). IVIg infusion was stopped, and treatment was started with intravenous clemastine fumarate (2 mg), corticosteroids (Di-Adreson-F, 25 mg), and NaCl 0.9% solution (500 mL). The condition of the patient improved within the next few hours, and she was discharged 24 hours later. Blood cultures and cultures from the immunoglobulin batch remained sterile. Serum tryptase and complement activation product concentrations during the anaphylactoid reaction were not increased in contrast to neutrophil-derived serum elastase concentrations, as monitored in serial blood samples during this episode (see Table E1 in this article's Online Repository at www.jacionline.org and data not shown). Reanalysis of preinfusion serum samples showed the presence of anti-IgA antibodies of the IgG_1 subclass. Two weeks after the incident and under appropriate monitoring, the patient received immunoglobulin subcutaneously (intramuscular immunoglobulin [IMIg] 16% solution) in gradually increasing dosages for 3 subsequent days.[18] Weekly administration of IMIg continued until she received IVIg without problems 2 months later, when anti-IgA had completely disappeared, probably through formation of IgA–anti-IgA complexes.[19,20]

Measurement of elastase, tryptase, and complement activation products

Detection of elastase, tryptase, and activation of C4, C3, and C1q-C4 complexes was performed as described previously in detail.[21-24]

Patient cohorts and blood sampling for DNA extraction

EDTA-anticoagulated blood samples were obtained from 52 additional adult patients given a diagnosis of hypogammaglobulinemia for DNA extraction. Patients were selected according to the international criteria for CVID.[25] In 33 patients the presence of antibodies against IgA had been assessed in serum taken before the initiation of IVIg infusions. In case of KD, blood was obtained from more than 200 pediatric patients, all treated with IVIg (2 g/kg, single dose) and oral aspirin within 10 days after the onset of fever. KD was defined by the presence of fever for 3 or more days, together with at least 4 of the 5 classical diagnostic criteria.[16,17] Additional blood samples were available for DNA extraction from 93 patients less than 18 years of age with ITP who had been treated with IVIg. The diagnosis of acute ITP was made according to the guidelines of the American Society of Hematology and the United Kingdom practice for management of acute childhood ITP.[26,27]

Informed consent was obtained from all parents of patients younger than 12 years and from all patients older than 12 years. Ethnicity was determined by means of self or parental ethnic identification. Healthy control subjects were unrelated adult white blood donors residing in the same geographic area. These studies were approved by the Medical Ethics Committee of the Academic Medical Center in Amsterdam and were performed in accordance with the Declaration of Helsinki.

mAbs and reagents

The following mAbs against human FcγRs were used: CD16 (anti-FcγRIII, clone 3G8 prepared as $F[ab']_2$ fragments) and CD32 (anti-FcγRII, clone IV.3; generous gifts from Dr Masja de Haas, Sanquin Research, Amsterdam, The Netherlands); CD16-phycoerythrin (anti-FcγRIII, IgG_{2a} isotype, clone CLB-FcR-gran/1, 5D2; Sanquin); CD32–fluorescein isothiocyanate (FITC; anti-FcγRII, IgG_1 isotype, clone KB61; DakoCytomation, Glostrup, Denmark); and anti-CD64–FITC (anti-FcγRI, IgG_1 isotype, clone 10.1; InstruChemie, Delfzijl, the Netherlands). Relevant isotype controls were obtained from Sanquin: isotype control IgG_{2a}-phycoerythrin (clone 713) and IgG_1-FITC (clone 203); anti-hIgG_1 (clone MH161, IgG_{2b},κ); anti-hIgG_2 (clone MH162, IgG_1,κ); anti-hIgG_3 (clone MH163, IgG_1,κ); anti-hIgG_4 (clone MH164, IgG_1,κ); and biotinylated rat anti-mouse κ-light chain (clone 226). Streptavidin-polyHRP (Sanquin) was used for the detection of hIgGs. Rabbit polyclonal anti-FcγRIIa was a generous gift from Dr Arthur Verhoeven.[28]

Isolation of neutrophils and PBMCs

Heparinized venous blood was collected from healthy donors and selected patients after obtaining informed consent and separated over a Percoll gradient (Amersham Biosciences, Uppsala, Sweden) into PBMCs as interphase and granulocytes with erythrocytes in the pellet. Neutrophils were purified by means of lysis of the erythrocytes with ice-cold isotonic NH_4Cl containing $NaHCO_3$, washed, and suspended in HEPES buffer (25 mmol/L HEPES, 123 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 10 mmol/L