Journal of Clinical Immunology (2018) 38:144–148
https://doi.org/10.1007/s10875-017-0466-7

ORIGINAL ARTICLE

A Severe Anaphylactic Reaction Associated with IgM-Class Anti-Human IgG Antibodies in a Hyper-IgM Syndrome Type 2 Patient

Yuki Tsujita[1] · Kohsuke Imai[1,2] · Kenichi Honma[1] · Chikako Kamae[1] · Takahiko Horiuchi[3] · Shigeaki Nonoyama[1]

Received: 6 November 2016 / Accepted: 22 November 2017 / Published online: 19 December 2017

Abstract

Purpose A 42-year-old man with hyper-IgM syndrome type 2 caused by activation-induced cytidine deaminase (AID) deficiency developed a severe anaphylactic reaction to intravenous immunoglobulin. The purpose of this study was to clarify the cause of the anaphylactic reaction of the patient.

Methods We measured IgM-class anti-human IgG and anti-human IgA antibodies in his serum by sandwich enzyme-linked immunosorbent assay (ELISA).

Results The sandwich ELISA assay revealed that serum from the patient, but not the controls, reacted to three different IgG products and purified human IgA. This indicated that the patient had IgM-class anti-human IgG and IgA antibodies in his serum, which associated with the anaphylactic reactions after the administration of IgG products. The anti-IgG antibody was likely to be the main cause of the reactions because an IgA-depleted IgG product also induced a severe reaction in this case and showed high absorbance in the ELISA system, similar to other IgG products containing more IgA.

Conclusions This is the first report of IgM-class anti-human IgG associated with an anaphylactic reaction to an IgG infusion. The anaphylactic reactions were very severe in this case, probably because IgM-class antibodies are potent activators of the complement pathway.

Keywords AID deficiency · anaphylactic reaction · IgM-class anti-human IgG antibody · hyper-IgM syndrome

Introduction

Immunoglobulin substitution with human IgG products is a well-established treatment to reduce the frequency of infections in primary immunodeficiency patients with antibody defects [1]. However, intravenous immunoglobulin (IVIG) administration leads to various side effects, such as headache, fever, and malaise in approximately 24% of patients with about 5% of infusions [2]. Among the adverse effects, anaphylactic reactions are rare, but most are severe and can be life-threatening.

Anaphylactic reactions to immunoglobulin products are known to be caused by hypersensitivity to the small amounts of IgA remaining in the IgG products [3–5]. Isolated IgA-deficient patients develop anti-human IgA antibodies after blood transfusion or administration of plasma products [6]. These anti-IgA antibodies are mainly IgG-class, although IgM and IgE-class anti-IgA antibodies have been described in some patients [5, 6]. The anti-IgA antibodies cause anaphylactic reactions to the administration of IgG products in isolated IgA-deficient patients through antigen (IgA) and antibody (anti-IgA) responses. However, anti-IgG antibodies have not been reported to cause anaphylactic reactions after administration of IgG products.

Here, we report the first case that IgM-class anti-human IgG antibodies are likely to cause anaphylactic reactions to IgG products.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10875-017-0466-7) contains supplementary material, which is available to authorized users.

✉ Yuki Tsujita
neige0118@gmail.com

[1] Department of Pediatrics, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-0042, Japan

[2] Department of Community Pediatrics, Perinatal and Maternal Medicine, Tokyo Medical and Dental University (TMDU), Tokyo, Japan

[3] Kyushu University Beppu Hospital, Beppu, Japan

Case

The patient was a 42-year-old man. He was introduced to our hospital because of recurrent pneumonia since childhood and hypoglobulinemia with elevated serum IgM. The results of laboratory tests revealed that IgG (< 0.03 g/l), IgA(< 0.03 g/l), and IgE (< 0.3 IU/ml) were undetectable and IgM was extremely high (10.62 g/l) in his serum. Complete blood count revealed a hemoglobin of 14.5 g/dl, platelet count 167,000/μl, and white blood cell count 5100/μl. Genomic analysis revealed a homozygous mutation in the activation-induced deaminase (*AID*) enzyme gene, an exon 3 c.335G > A, p.R(Arg)112(His) missense mutation that was identical to one previously identified in his sister who had been diagnosed with AID deficiency. The patient was diagnosed as having hyper-IgM syndrome due to AID deficiency.

After diagnosis, the patient began gamma globulin replacement therapy. However, severe anaphylactic shock occurred after only a small amount (less than 1.0 ml) of IgG was infused. He recovered with the administration of oxygen, intravenous epinephrine, and steroids. One month later, he was admitted to our hospital, and the administration of a different immunoglobulin product was attempted. Because he had no serum IgA, it was considered that the patient had antibodies against IgA, which had caused the previous anaphylactic reaction. We, therefore, used a freeze-dried ion exchange resin-treated immunoglobulin product, which is IgA-depleted and has the lowest level of IgA among all of the human IgG products available in Japan. Methylprednisolone and chlorpheniramine maleate were administered before starting IVIG. However, the patient developed dyspnea, conjunctival injection, hypoxia, and tachycardia just after the administration of 1.0 ml of the product. The transfusion was immediately terminated. After administration of oxygen and additional methylprednisolone, he gradually recovered from the anaphylactic reaction.

In this patient, both IgG and IgA were absent in the serum. Therefore, we hypothesized that IgG was recognized by his immune system as a foreign antigen as well as IgA, and we examined if the patient had antibodies against IgG and IgA by enzyme-linked immunosorbent assay (ELISA). We further examined whether the anti-IgG and anti-IgA antibodies were IgM-class because the patient had only IgM but no IgG or IgA due to AID deficiency. We also tried to find out whether IgM-class antibody reacts to IgM itself or not to confirm the specificity of the assay. Although his IgE level was undetectable in the serum, we also examined if the patient had IgE-class anti-IgG and anti-IgA because anaphylactic reactions might have been caused by a subtle amount of IgE.

Materials and Methods

Elisa

In a sandwich ELISA to detect IgM-class antibodies to human IgG, we used three types of immunoglobulin products as coating antibodies: pH 4-treated, freeze-dried pepsin-treated, and freeze-dried ion exchange resin-treated. Ninety-six-well plates (Falcon 3072, BD Biosciences) were coated with each diluted preparation (0.5 μg/ml), and sera from the patient or a normal control was added. Each serum sample was diluted to contain the same concentration of IgM (approximately 1000 ng/ml). The plates were then treated with biotin-conjugated goat anti-human IgM (B1265, Sigma). Streptavidin-horseradish peroxidase was added, and the color reaction was developed with tetramethylbenzidine. Finally, color development was stopped with 2 M sulfuric acid, and absorbances at 450 nm were read with an ELISA plate reader.

In the sandwich ELISA to detect IgM-class antibodies to human IgA and human IgM, we used purified human IgA (16–16-090701, Athens Research and Technology) and IgM (401,799, Calbiochem) as the coating antibodies, and followed the procedure as described above.

In the sandwich ELISA to detect IgE-class antibodies to human IgG and IgA, we used immunoglobulin product and purified human IgA as the coating antibody, and HRP conjugated goat anti-human IgE antibody (A80-108P-33, Bethyl Laboratories) as the secondary antibody.

The levels of serum IgG, IgA, IgM, and IgE of two healthy controls were 12.9, 2.53, 1.58, and 91.4 IU/ml in the control 1, and 13.4, 2.20, and 0.79 g/l and 213.9 IU/ml in the control 2, respectively. Human purified IgM (401799, Calbiochem) was used as background in all ELISA experiments, and O.D. value are shown by subtracting the value of background from each value.

Measurement of Human C3a and C5a

Human C3a (HK354, Hycult Biotech) and C5a (HK359, Hycult Biotech) sandwich ELISA kits were used in accordance with the manufacturer's protocols.

Statistical Analysis

All values were statistically analyzed by Welch's *t* test, and expressed as means and standard errors of the mean.

Results

The sandwich ELISA detected IgM-class anti-IgG and anti-IgA antibodies in the patient's serum. The optical density (OD) values of the patient and healthy controls' serum were

obtained by subtracting a background value from the each value. The OD values of the patient's serum were positive for all three different IgG products (X, Y, and Z) that were used as coating antibodies (Fig. 1a). The OD values were as follows, 0.94 ± 0.08 for IgG product X, 0.53 ± 0.02 for product Y, and 0.91 ± 0.11 for product Z. These values were significantly higher than two normal controls (Fig. 1a; product X ($p = 0.0009$ and $p = 0.0057$), product Y ($p = 0.0008$ and $p = 0.0016$), and product Z ($p = 0.0097$ and $p = 0.0078$)). The OD values of the normal controls were considered to be negative. These results indicated that anti-IgG antibody was present in the patient's serum and absent in the controls' serum. Because we used anti-IgM antibody as the secondary antibody in this ELISA system, the patient's anti-IgG antibody was considered to be IgM-class.

Since the IgG products used as the coating antibody contained a subtle amount of IgA, it is possible that anti-human IgA antibody in the patient's serum reacted to the remaining IgA, and the OD values may have been falsely positive. However, the amounts of remaining IgA were different among the three IgG products (i.e., the pH 4-treated reagent contained 953.0 mg/l of IgA, the freeze-dried pepsin-treated reagent contained 6130.0 mg/l of IgA, and the freeze-dried ion exchange resin-treated reagent contained 1.6 mg/l of IgA (Fig. 1a)), but the OD values of these three IgG products did not correlate with the amounts of remaining IgA in each IgG product [7]. This observation indicates that the ELISA system detected the anti-IgG rather than the anti-IgA in the patient's serum.

In the sandwich ELISA to detect anti-human IgA, the OD of the patient's serum was positive (0.78 ± 0.02), which was significantly higher than the two normal controls ($p = 0.0002$ and $p = 0.0035$, Fig. 1b). The OD values of the two normal controls were low and similar to the background, and thus considered to be negative (Fig. 1b). This observation indicates that anti-human IgA antibody was also present in the patient's serum, but absent and undetectable in the two healthy controls' sera. Because anti-human IgM was used as the secondary antibody in this ELISA system, the anti-IgA antibody was also IgM-class. Neither IgE anti-human IgG nor IgE anti-human IgA was detected by sandwich ELISA (Supple Fig. 1A and B). We also performed the ELISA to detect IgM anti-IgM in serum, and IgM anti-IgM is extremely low level in both the patient and controls' serum in that ELISA system (Supple Fig. 1C). The result indicated that anti-human IgM antibody was absent in the patient and two healthy controls' sera.

All together, these results revealed that the patient had both IgM-class anti-human IgG antibody and IgM-class anti-human IgA antibody in his serum.

IgM anti-IgG/IgA antibodies seem to recognize IgG products as foreign antigens. This reaction may cause activation of the complementary system, and produce complement splits. Therefore, we measured C3a and C5a in the sera of the patient and the control. C3a and C5a levels in the patient serum were founded to be higher than those of the control. The concentration of C3a was as follows, 3.95 μg/ml in the patient and 0.137 μg/ml in the healthy control, and the concentration of

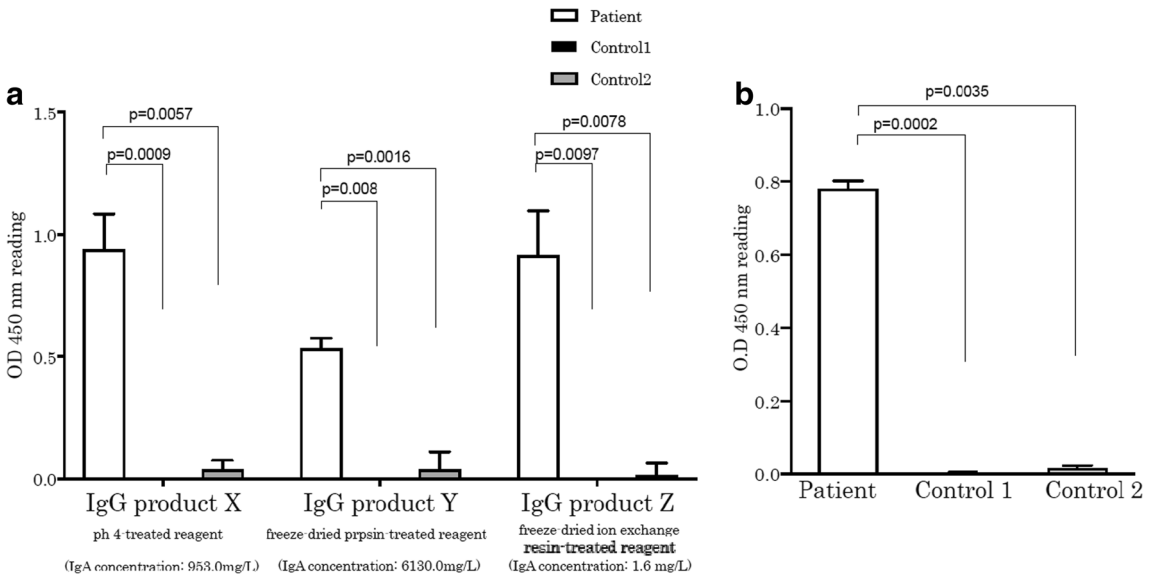

Fig. 1 Detection of IgM-class anti-human IgG and anti-human IgA in the serum by sandwich ELISA. Data indicate spectrophotometric absorption at 450 nm. Data shown in the figures are the mean ± standard error obtained in one experiment performed in triplicate. **a** IgM-class anti-human IgG was measured using three different IgG products (X, Y, and Z) as coating antibodies. IgG product X was a pH 4-treated product, Y was a freeze-dried pepsin-treated product, and Z was a freeze-dried ion exchange resin-treated product. **b** IgM-class anti-human IgA was measured using purified human IgA as the coating antibody

C5a was as follows, 34.7 ng/ml in the patient and 22.1 ng/ml in the healthy control.

Discussion

This study demonstrated the possibility that the anaphylactic reaction to IgG products observed in this patient was caused by IgM-class anti-human IgG antibodies. We suppose that the anaphylactic reaction was associated with immunological reactions between the patient's anti-IgG and the transfused IgG, and reactions between the patient's anti-IgA and a subtle amount of IgA contained in the IgG products. An anti-IgG antibody causing an anaphylactic reaction to IgG products has never been reported, although IgG-class anti-human IgA antibodies are well known to cause anaphylactic reactions to blood products in IgA-deficient patients [3–6]. The probable reason that anti-IgG antibody production has never been observed is that most patients with hypogammaglobulinemia have low but detectable levels of IgG, including patients with X-linked agammaglobulinemia caused by conditions such as Bruton tyrosine kinase deficiency, CD40L deficiency, and common variable immunodeficiency [8]. In contrast, in most AID-deficient patients, serum IgG is completely absent and undetectable [8, 9], and it is likely that IgG is recognized as a foreign antigen by the patients' immune systems.

IgM-class anti-human IgA antibody was also present in the patient's serum, which was possibly also involved in the anaphylactic reaction to the IgG products. However, we consider that the anti-IgG antibody was the main cause of the reactions because only 1.0 ml of the IgA-depleted IgG product (the freeze-dried ion exchange resin-treated IgG reagent) that contains only a tiny amount of IgA also gave rise to a similarly severe reaction as to the other IgG product.

IgM, as well as IgG, are known to cause anaphylactic reactions [10]. Unlike IgG, IgM is a pentamer with five Fc regions, and it is a highly potent activator of the complement system. Therefore, IgM induces stronger reactions against antigens than IgG. Therefore, since the patient had IgM-class anti-IgG antibodies, it seems to be natural that he manifested an extremely severe anaphylactic reaction to small amounts of IgG. We found that C3a and C5a levels in the patient serum are higher than those of the control, which might suggest that complement activation tend to be induced in the patient serum. Although IgE is well known to cause anaphylaxis via activation of mast cells, IgE-type anti-IgG and anti-IgA antibodies were not detected in the patient's serum. Also, the patient's B cells fail to produce IgE in vitro by anti-CD40 and IL-4 stimulation by the method previously described [11, data not shown]. Thus, we believe that IgE-type antibodies were not the main cause of anaphylactic reactions in this patient. However, there is a possibility that trace amounts of IgE class anti-IgG and/or IgE class anti-IgA, which are not detectable by ELISA, are present and may also be involved in the induction of the series of anaphylactic reactions in this patient.

We tried to find out how the patient could have developed IgM anti-IgG antibodies since he had never received a blood transfusion. The antibodies is likely to have developed after receiving gamma globulin when he was treated for recurrent pneumonia during childhood, because IgG products are sometimes used as a treatment for severe pneumonia with antibiotics in Japan. Alternatively, these may be natural antibodies, similar to that cause anaphylactic reactions in some IgA-deficient patients following their first infusion of a blood product [5]. We have 16 AID-deficient patients in Japan [11, 12 and unpublished data], and none of them have had anaphylactic reactions to IgG products. Although his sister is a hyper IgM type2 patient with the same *AID* mutation, she has been able to receive regular IVIG treatment without any anaphylactic reactions. Therefore, we consider that not all the AID-deficient patients have IgM-type natural antibodies against IgG and IgA. Thus, his IgM anti-IgG and anti-IgA seem to have been produced after administration of immunoglobulin products for treatment of severe pneumonia in his childhood. However, we could not find it out that he had received an IgG product in his childhood because his old records are missing.

The patient has been taking prophylactic antibiotics (trimethoprim/sulfamethoxazole) and has been treated with antibiotics when he suffered from bacterial infection. Although he is keeping in a relatively good condition, we believe that he needs periodical IgG administration. However, we are not able to try the gamma globulin infusion therapy because of the results found in this study.

This is the first report that indicates the possibility that IgM-class anti-human IgG is associated with an anaphylactic reaction to an IgG infusion. Physicians should be aware of the possibility of anaphylactic reactions caused by IgG products when patients lack serum IgG.

Acknowledgments This work was supported in part by the Ministry of Defense, Japan; the Research on Measures for Intractable Diseases Project (H29-013, H26-037, H23-012): matching fund subsidy from the Ministry of Health, Labour and Welfare, Japan; the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan (17913039, 26293250, 15 K09640); the Practical Research for Rare/Intractable Diseases from Japan Agency for Medical Research and Development, AMED; the Japan Foundation for Pediatric Research; the Jeffrey Modell Foundation; and the Kawano Masanori Memorial Public Interest Incorporated Foundation for Promotion of Pediatrics.

Authors' Contributions KI and SN contributed to study conception and design; YT wrote the manuscript and SN and KI reviewed the manuscript; KH performed the ELISA to detect the IgM-type anti IgG antibody; YT preformed the ELISA to detect the IgM-type anti-IgA, IgM-type anti-IgM, IgM-type anti-BSA, IgE-type anti-IgG, and IgA antibody; TH designed assays to measure C3a and C5a and YT performed those assays;

KI, KH, CK, and SN contributed to interpretation of data; YT performed the statistical analysis; SN provided clinical samples and data; and all authors reviewed the manuscript and approved the final version.

Compliance with Ethical Standards The experiments in this study were done after written informed consent had been obtained and according to the permits issued by the Institutional Review Board of National Defense Medical College (permit number 1275).

Research Involving Human Participants All procedures performed in the study were in accordance with the Helsinki principles, and the study was approved by the Institutional Review Board of the National Defense Medical College.

Conflict of Interest The authors declare that they have no conflicts of interest.

References

1. Orange JS, Hossny EM, Weiler CR, et al. Use of intravenous immunoglobulin in human disease: a review of evidence by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology. J Allergy Clin Immunol. 2006;117(4 Suppl):S525–53. https://doi.org/10.1016/j.jaci.2006.01.015.
2. Eijkhout HW, van Der Meer JW, Kallenberg CG, et al. The effect of two different dosages of intravenous immunoglobulin on the incidence of recurrent infections in patients with primary hypogammaglobulinemia. A randomized, double-blind, multicenter crossover trial. Ann Intern Med. 2001;135(3):165–74. https://doi.org/10.7326/0003-4819-135-3-200108070-00008.
3. Wells JV, Buckley RH, Schanfield MS, Fundenberg HH. Anaphylactic reactions to plasma infusions in patients with hypogammaglobulinemia and anti-IgA antibodies. Clin Immunol Immunopathol. 1977;8(2):265–71. https://doi.org/10.1016/0090-1229(77)90116-7.
4. Vyas GN, Perkins HA, Fudenberg HH. Anaphylactoid transfusion reactions associated with anti-IgA. Lancet. 1968;2(7563):312–5.
5. Sandler SG, Mallory D, Malamut D, Eckrich R. IgA anaphylactic transfusion reactions. Transfus Med Rev. 1995;9(1):1–8. https://doi.org/10.1016/S0887-7963(05)80026-4.
6. Burks AW, Sampson HA, Buckley RH. Anaphylactic reactions after gamma globulin administration in patients with hypogammaglobulinemia. Detection of IgE antibodies to IgA. N Engl J Med. 1986;314(9):560–4. https://doi.org/10.1056/NEJM198602273140907.
7. Kitamura K, Mikami Y. Analysis of the physicochemical and biochemical property of various intravenous immunoglonbulin preparations. Biotherapy. 2002;16:467–76. (Article in Japanese)
8. Ochs HD, Smith CIE, Puck JM. Primary immunodeficiency diseases. A molecular and genetic approach. Third edition, NY, USA, 2014. p. 299–365.
9. Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). Cell. 2000;102(5):565–75. https://doi.org/10.1016/S0092-8674(00)00079-9.
10. Fabry U, Körholz D, Jürgens H, Göbel U, Wahn V. Anaphylaxis to L-asparaginase during treatment for acute lymphoblastic leukemia in children—evidence of a complement-mediated mechanism. Pediatr Res. 1985;19(4):400–8. https://doi.org/10.1203/00006450-198519040-00017.
11. Zhu Y, Nonoyama S, Morio T, Muramatsu M, Honjo T, Mizutani S. Type two hyper-IgM syndrome caused by mutation in activation-induced cytidine deaminase. J Med Dent Sci. 2003;50(1):41–6.
12. Minegishi Y, Lavoie A, Cunningham-Rundles C, Bédard PM, Hébert J, Côté L, et al. Mutations in activation-induced cytidine deaminase in patients with hyper IgM syndrome. Clin Immunol. 2000;97(3):203–10. https://doi.org/10.1006/clim.2000.4956.