ASSOCIATION BETWEEN ANTI-IgA ANTIBODY AND DEVELOPMENT OF ADVERSE REACTIONS IN IMMUNODEFICIENT PATIENTS UNDER IMMUNOGLOBULIN THERAPY

A. Roohi,[*] *A. Aghamohammadi,*[**] *F. Shokri* [*]

[*]*Department of Immunology, School of Public Health,* [**]*Department. of Pediatrics, Clinic of Immunology and Allergy, School of Medicine, Tehran University of Medical Sciences*

ABSTRACT

Background: Mild to severe post-transfusion adverse reactions have been reported in a proportion of immunodeficient patients receiving intravenous immunoglobulin (IVIg). Anti-IgA antibody has been proposed to be associated with development of such reactions.

Objective: To assess the association between anti-IgA levels of different isotypes and adverse reactions induced in immunodeficient patients following infusion of IVIg.

Methods: In this study, IgM, IgG and IgE anti-IgA antibodies were measured by an indirect ELISA method in serum samples from 31 patients with a variety of immunodeficiencies receiving IVIg and 24 normal individuals.

Results: Anti-IgA antibody of all three isotypes were detectable in all sera. However, when presented as proportion of total IgA (anti -IgA/total IgA)IgG, IgE isotypes are significantly higher in patients with adverse reactions compared to those without reactions or normal subjects.

Conclusion: Both IgG and IgE anti-IgA antibodies may contribute to the development and severity of adverse reactions in some patients receiving IVIg.

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Key Words • Passive immunization • immunodeficiency • common variable immunodeficiency • immunoglobulin

Introduction

Primary antibody deficiency represents a group of immunodeficiency disorders with diverse etiologic agents and distinct clinical entities. The most common form of primary antibody deficiency is selective IgA deficiency (IgAD)[1] with heterogeneous clinical manifestations.[2] This disease, together with common variable immunodeficiency (CVID), are considered as polar ends of the immunoglobulin (Ig) deficiency spectrum. [2-4] In addition to IgA, other isotypes of Ig may also be differentially affected in CVID. There are many reports of IgAD conversion to CVID.[5-7] These findings propose a common origin for both disorders. [2,8] Although in both conditions, and also other primary antibody deficiencies, Ig replacement is the major treatment,[4,9] mild to severe side effects may be induced in a proportion of patients.[10,11] Immunoglobulin aggregates[4] and anti-IgA antibodies have been proposed as causative agents for such reactions. [10,12] The

Correspondence: *F. Shokri, M.D., Department of Immunology, School of Public Health, Tehran University of Medical Sciences, P.O. Box:6446-14155, Tehran, Iran. Tel:+9821-6112406-9, Fax:+9821-6462267*

isotype of anti-IgA antibody, however, remains a matter of controversy. [13-15] In the present study, IgM, IgG and IgE anti-IgA antibodies were measured in sera collected from Iranian patients with different primary antibody deficiencies and the results were compared in different groups of patients based on total IgA levels and development of adverse reactions.

Materials and Methods

Subjects:

Sera were obtained from 31 immunodeficient patients, including common variable immunodeficiency (CVID: n=10, age: 2-27 years, male=7, female=3), selective IgA deficiency (IgAD: n=6, age=10-16 years, male=4, female=2), X-linked agammaglobulinemia (X LA: n=5, age=7-13 years, male=5) and Ataxia telangiectasia (AT: n=10, age=8-16 years, male=5, female=5), who were under IVIg therapy. Major clinical symptoms observed in most of the patients were related to respiratory and gastrointestinal infections and chronic otitis media. All samples were collected from the patients seen in the Clinic for Immunology and Allergy, Children Medical Center, Tehran University of Medical Sciences, just before the regular infusion of Ig preparation. The patients were classified into a "IgAD+CVID" group and "others" and adverse reactions were recorded in each group (Table 1). Ratings of adverse reactions were performed according to Misbah et al.,[11] Mild adverse reactions included headache, flushing, low backache, nausea and wheezing. Serum samples from 24 normal volunteers served as controls. Samples were stored at -20°C until biochemical analysis was performed.

Measurement of IgM, IgG and IgE anti-IgA antibodies:

IgM, IgG and IgE anti-IgA antibodies comprising different isotypes of anti-IgA antibody were measured by an indirect ELISA, as reported elsewhere[14-16] with some modifications. Briefly, ELISA plates (Nunc, Denmark) were coated in duplicate with 10 μg/ml in PBS of affinity purified IgA paraprotein isolated from serum of a patient with multiple myeloma (MM 38). Following 90 min incubation at 37°C, the plates were washed with PBS containing 0.05% tween 20 (PBS/T) (Sigma, USA) and incubated with serum samples prediluted to 1/100 in PBS/T. The plates were finally washed with PBS/T and incubated with appropriate dilutions of isotype-specific HRP-conjugated sheep anti-human IgM, IgG or IgE (Sigma, USA). Unbound conjugate molecules were washed and the color was developed by addition of O-phenylenediamine (OPD, Sigma, USA) substrate and optical density (OD) was recorded by a multi-scan ELISA reader (Organon Teknika, Holland) at 492 nm.

Measurement of total IgA:

Total IgA was qualitatively measured in serum by a direct ELISA method. Briefly, three dilutions of each sample (1/10000, 1/30000, 1/100000) were prepared in PBS and coated in duplicate in a polystyrene ELISA plate (Nunc, Denmark). Following 90 min of incubation at 37°C, the plates were extensively washed with PBS/T and incubated with appropriate dilution of HRP-conjugated sheep anti-human IgA (Sigma, USA). Finally, the plates were washed and the reactions revealed by addition of OPD (Sigma, USA) and ODs were measured at 492 nm by a multiscan ELISA reader.

Statistical analysis:

Comparison of parameters was performed using one-way analysis of variance with Tukey's HSD post-hoc comparisons. All calculations were performed using the package SPSS for Windows Release 9. P values of less than 0.05 were considered significant.

Results

All patients were classified based on their

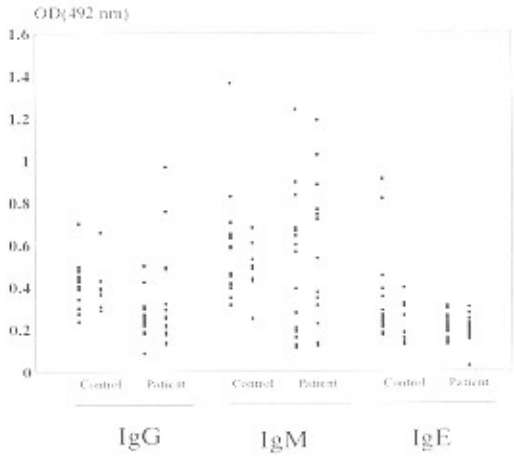

Figure 1: Serum levels of IgM, IgG and IgE anti-IgA in immunodeficient patients and normal subjects

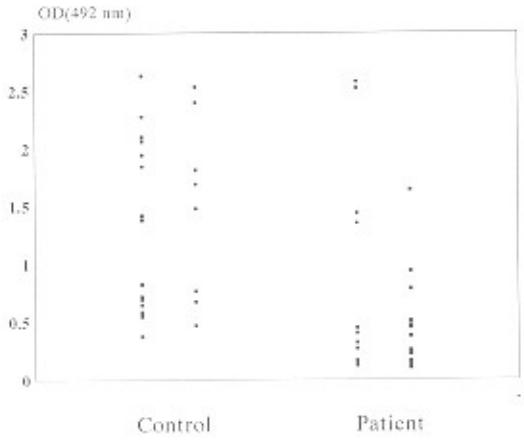

Figure 2: Serum levels of IgA in immunodeficient patients and normal subjects

disease status and development of adverse reactions after receiving IVIg (Table 1). Antibodies to IgA were found in serum of all patients and normal individuals (Fig. 1). Distribution of titers of total IgA in all sera is shown in Figure 2. Comparison of raw OD data of different isotypes of anti-IgA between all groups of patients and normal individuals revealed no elevation in titer of antibodies in patients. However, if presented as the proportion of total IgA (anti-IgA/total IgA), IgG, IgE, but not IgM anti-IgA antibodies were significantly higher in patients with adverse reactions compared to those without reactions. ($P < O.O5$). Comparison between patients and normal subjects revealed significant elevation of all isotypes in both groups of patients ($P < O.O1$) (Table 2). When CVID + IgAD patients were compared with other patients, no significant differences were observed for any of the isotypes, though both groups of patients displayed a significantly higher proportion of IgG ($P < O.OO1$), IgE ($P < O.OO1$) and IgM ($P < O.OO1$) anti-IgA compared to normal subjects.

Table 1: Frequency of adverse reaction in different groups of immunodeficient patients

Patients	Adverse reactions
CVID + IgAD (n=16)	Mild (n=10)
Others (n=15)	Mild (n=7)

CVID: Common variable immunodeficiency, **IgAD:** Selective IgA deficiency

Discussion

A wide range of post-transfusion adverse reactions have been reported in immunodeficient patients receiving IVIg. [10,12,17] Development and severity of such reactions have been demonstrated to be associated with anti-IgA antibody.[10,12] Anti-IgA antibody was first detected by agglutination method, using red blood cells sensitized with monoclonal IgA paraproteins.[18] The use of ELISA method improved assay sensitivity and facilitated detection and measurement of all isotypes of anti-IgA antibodies. [14,19] Application of this methodology resulted in identification of anti-IgA isotypes associated with adverse reactions as well as detection of these antibodies in a large number of normal individuals. [13] Variability of the results, however, has prompted some investigators to use polyclonal

Table 2: Comparison between different isotypes of anti-IgA antibody in immunodeficient patients and normal individuals

parameter	IgE anti-IgA/total IgA	IgG anti-IgA/total IgA	IgM anti-IgA/total IgA
CVID + IgAD (n=16)	0.96 (0.60)	1.42 (1.3)	1.23 (0.65)
Other patients (n=15)	0.79 (0.65)	0.96 (0.64)	1.91 (1.58)
With adverse reaction (n=17)	1.09 (0.6)	1.4 (0.98)	1.78 (1.24)
Without adverse reaction (n=14)	0.63 (0.54)	0.74 (0.59)	1.33 (1.26)
Normal subjects (n=24)	0.37 (0.51)	0.42 (0.27)	0.56 (0.34)

*The figures represent mean and standard deviation (SD) of OD values for each parameter.

IgA instead of monoclonal IgA preparations,[14] but no significant differences were found.[13,14,20] Comparative measurement of the major isotypes of anti-IgA antibody, particularly IgE, has not been extensively studied to explore their role in the development of adverse reactions in patients under IVIg therapy. Our results demonstrate the presence of all isotypes of anti-IgA in normal individuals, a finding already reported by others.[13] These antibodies were shown to have Fab specificity.[13] However, our unexpected finding was that the levels of these antibodies in normal individuals were comparable and to some extent even higher than those of the patients (Table 2). Although no standard anti-IgA sample with known concentrations of anti-IgA isotypes is available to extrapolate concentrations from a standard curve, since all samples were run together under the same condition, raw OD data could be used and compared and the results may be considered as valid and reliable. The important aspect which needs to be considered in such studies, and has been overlooked by many investigators, is the fact that most Ig isotypes are significantly decreased in primary antibody deficient patients.[9] Thus, detection of low levels of anti-IgA antibody in these patients is a reflection of generalized hypogammaglobulinemia observed in such patients. To be able to compare the results between normal individuals and antibody deficient patients, total Ig levels should be taken into consideration. This, together with the fact that anti-IgA antibody level has been repeatedly found to be inversely proportional to total serum IgA level,[14,15,21] suggest that the results of anti-IgA antibody should be presented as a function of total IgA levels (anti-IgA/total IgA) to achieve more precise total IgA comparative results. Indeed, these considerations have allowed distinction not only between the patients and normal individuals, but also between the patients with or without adverse reactions (Table 2). Accordingly, all isotypes of anti-IgA, particularly IgG and IgE, were found to be significantly increased in patients having adverse reactions compared to those without such reactions or normal individuals. Collectively, contrary to some reports,[2] our findings are in agreement with those of others indicating the involvement of IgG [10,15,21] and

IgE [17] anti-IgA antibody in the development of adverse reactions in those receiving IVIg therapy. Measurment of anti-IgA isotypes at different time intervals in immunodeficient patients with adverse reactions may help illuminate the role of these antibodies in the development and severity of such reactions.

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