

IgG Anti-IgE Autoantibodies in Visceral Leishmaniasis

AM Atta/⁺, MLB Sousa-Atta, A D'Oliveira*, RP Almeida*, MI Araújo*, EM Carvalho*

Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal da Bahia, Rua Barão de Geremoabo s/n°, Campus Ondina, 40171-970 Salvador, BA, Brasil *Serviço de Imunologia, Hospital Universitário Prof. Edgar Santos, Salvador, BA, Brasil

Procedures for IgG depletion in visceral leishmaniasis (VL) and schistosomiasis sera using Sepharose-protein G beads also deplete IgE. In this study, the presence of IgG anti-IgE autoantibodies in sera from patients with VL (n = 10), and hepatic-intestinal schistosomiasis (n = 10) and from healthy individuals (n = 10) was investigated. A sandwich ELISA using goat IgG anti-human IgE to capture serum IgE and goat anti-human IgG peroxidase conjugate to demonstrate the binding of IgG to the IgE captured was performed. VL sera had higher titers (p < 0.05) of IgG anti-IgE autoantibodies (OD = 2.01 ± 0.43) than sera from healthy individuals (OD = 1.35 ± 0.16) or persons infected with Schistosoma mansoni (OD = 1.34 ± 0.18). The immunoblotting carried out with eluates from Sepharose-protein G beads used to deplete IgG from these sera and goat anti-human IgE peroxidase conjugate, showed a similar pattern of bands, predominating the 75 kDa ε-heavy chain and also polypeptides resulting from physiological enzymatic digestion of IgE. A frequent additional band immediately above 75 kDa was observed only in VL sera.

Key words: IgE - IgG anti-IgE - protein G - visceral leishmaniasis - schistosomiasis mansoni

Immunoglobulin E is an isotype of antibody that has Fc receptors of different affinity on basophils, mast cells, eosinophils, macrophages and platelets. The immune reaction of IgE fixed on these cells with the corresponding antigen can release bioactive cell mediators as histamine, sulfidopeptide leukotrienes, prostaglandin D₂ or platelet-activating factor, causing systemic or local inflammatory reactions. The involvement of IgE in host immune response to parasite antigens was showed immediately after its immunochemical identification in 1969, when high concentration of this protein in serum was obtained from persons suffering of helminthiasis (Bennich et al. 1969, Kojima et al. 1972). The recent studies on the dichotomy of immune response in Th1 and Th2 (Coffman et al. 1991), which is related to the pattern of cytokines produced during antigenic stimulation, have shown that IgE is the mainly antibody induced by IL-4 and IL-13 during a Th2 immunologic event (Romagnani 1998, 2000, Hunter & Reiner 2000). This fact has provoked the interest in the research of this isotype of immunoglobulin in several diseases associated to a Th2 immunological pattern such as systemic lupus erythematosus and viral and parasitic infections (Elkayam et al. 1995, Mazza et al. 1995, Atta et al. 1998). A common pitfall in serum IgE immunoassays to diagnosis infections by protozoa or helminth, also present in IgM tests, corresponds to false negative results originated by serologic competition of IgG antibodies, generally present in greater concentration, and affinity for common epitopes on target antigens. In order to solve this problem, procedures of serum IgG depletion have been advised, employing anti-human IgG antibodies obtained through immunization of different species, followed by elimination of the immune precipitates formed after absorption by centrifugation.

Staphylococcus protein A, *Streptococcus* protein G or anti-IgG antibodies have also been used as ligands in experiments of serum IgG depletion after conjugation with inert particles as Sepharose beads.

Recently, when we investigated the occurrence of IgE anti-*Leishmania chagasi* antibodies in sera from patients with visceral leishmaniasis (VL), and also IgE anti-*Schistosoma mansoni* antibodies in sera from individuals with schistosomiasis, we observed that the use of Sepharose-protein G for the depletion of IgG caused significant fall in sensitivity of the specific immunoassays when the results obtained were compared with those derived from the use of RF-Absorbent (Behring Diagnostics, USA), a solution of purified anti-human IgG antibodies (Sousa-Atta et al. 1999). Considering that IgG anti-IgE autoantibodies have been demonstrated in healthy individuals and in patients with asthma, autoimmune and parasite diseases (Inghanas et al. 1981, Quinti et al. 1986, Gruber et al. 1988, Scheuer et al. 1991), and complexed IgG anti-IgE autoantibodies were documented in sera from Crohn's disease patients and from patients with rheumatoid arthritis (Huber et al. 1998, Millauer et al. 1999), the aims of this study was to evaluate the levels of complexed IgG anti-IgE autoantibodies in sera from VL and from with hepatic-intestinal schistosomiasis mansoni patients and also to investigate if treatment of human serum with Sepharose-Protein G causes IgE depletion through the sequestration of immunocomplexes IgG-IgE by the beads.

MATERIALS AND METHODS

Sera - Sera from 10 VL and 10 schistosomiasis mansoni patients were obtained from persons with clinical and laboratory diagnosis of these parasitic diseases, all assisted by the staff of the Immunology Service of the Hospital Universitário Professor Edgar Santos. Control sera were from 10 healthy individuals having negative serology for rheumatic and infectious diseases (American trypanosomiasis, viral hepatitis and syphilis).

Methods - The immunoassay to detect complexed IgG anti-IgE autoantibodies was an ELISA of immunocapture of IgE, developed on polystyrene microplates containing wells covered with goat IgG anti-IgE (Sigma Chemical Co., USA) and a goat IgG anti-human IgG peroxidase conju-

This work was supported by Pronex (grant no. 4196086200).

⁺Corresponding author. Fax: +55-71354.3862. E-mail: ajatta@ig.com.br

Received 5 March 2001

Accepted 3 October 2001

gate, from the same source, to demonstrate the immunocomplexes IgG-IgE captured. Briefly, the immune reaction to capture IgE was performed incubating 100 μ l of human sera diluted at 1/6 in 50 mM Tris-HCl (pH 7.5) buffered-saline containing 1% bovine serum albumine and 0.05% Tween 20 for 1 h at room temperature, while the reaction with 100 μ l of the diluted conjugate was developed in the same conditions, after wash of the wells. The reactions were revealed with hydrogen peroxide plus OPD (*ortho*-phenylenediamine) during 30 min, stopped with 2N HCl and determined at 492-600 nm in a DIAMEDIX BP-12 Microassay apparatus.

To demonstrate the sequestration of complexed IgG anti-IgE by protein G, three sera from each group under study were diluted at 1/5 in PBS containing 25% of Sepharose-protein G (Pharmacia Biotech, Uppsala, Sweden) and incubated during 15 min at room temperature. After repeated wash by centrifugation with PBS, the beads were treated with SDS-polyacrylamide gel electrophoresis sample buffer containing 2-mercaptoethanol to elute the captured material, followed by electrophoresis on a 10% acrylamide minigel. The polypeptides fractionated were transferred by electrophoresis to a PVDF membrane (Immobilon, Millipore, and USA) and analyzed for IgE by incubation with goat anti-human IgE peroxidase conjugated (Sigma Chemical Co., USA). After new wash, the immune reactions were revealed incubating the membrane with hydrogen peroxide plus DAB (3,3'-diaminobenzidine), as usually.

Serum IgE concentration was determined by ELFA using the Vidas 30 immunoanalyzer from biolabMeri  ux.

Statistical analysis were performed through the Primer PC statistic program using a non-parametric test U of Mann-Whitney.

RESULTS

Complexed IgG anti-IgE autoantibodies were detected in all sera studied. The results of the immunoassays to detect these immune complexes are presented in the Fig. 1. While schistosomiasis and normal sera presented similar titers, sera from VL patients had higher levels of complexed IgG anti-IgE when compared to these groups, as assessed by the U test of Mann-Whitney ($p < 0.05$). There was no correlation between IgE concentration and complexed IgG anti-IgE level in serum ($p > 0.05$). Patients from schistosomiasis group with serum IgE concentration of 97 IU/ml or 5800 IU/ml presented similar levels of complexed IgG anti-IgE autoantibodies.

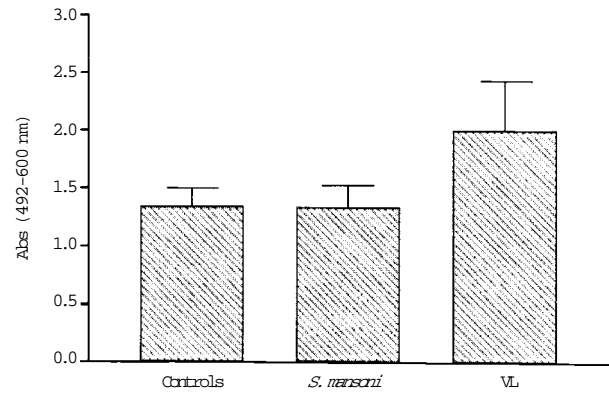


Fig. 1: IgG anti-IgE autoantibodies in sera from healthy individuals (controls) and from visceral leishmaniasis (VL) and schistosomiasis mansoni (*Schistosoma mansoni*) patients

Immunochemical analysis carried out with material eluted from batching experiments to deplete IgG from these sera evidenced significant capture of IgE immunoglobulin during IgG binding to Sepharose protein G beads, as demonstrated by intense brown colored band on PVDF membrane, with molecular weight corresponding to 75 kDa IgE heavy chain in all sera analyzed, inclusive from healthy controls. Other peptides of different weights that probably correspond to the products of physiologic degradation of this immunoglobulin were also observed (Fig. 2). However, only visceral leishmaniasis sera presented an additional polypeptide with molecular weight immediately above 75kDa-heavy chain.

DISCUSSION

The observations here reported indicated that complexed IgG anti-IgE might be detected in high concentration in sera from VL patients. These immune complexes are not increased in sera from patients suffering from hepatic-intestinal schistosomiasis mansoni, and their titers were not correlated with the elevated concentration of serum IgE presented by some infected patients. On the other hand, sera from schistosomiasis group presented the same reactivity pattern for IgG anti-IgE observed in sera from healthy controls, which had IgE concentration below 150 IU/ml. This argues against the participation of polyclonal B-cell activation in the production of IgG anti-IgE autoantibodies and indicates that the biological significance of

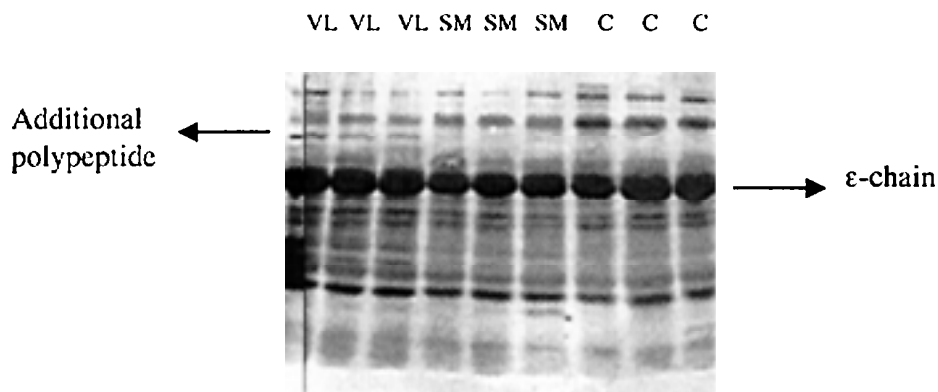


Fig. 2: serum IgE depletion after treatment with Sepharose-protein G of sera from patients with visceral leishmaniasis (VL), schistosomiasis mansoni (SM) and clinically health individuals (controls/C)

the increased levels of IgG anti-IgE autoantibodies needs to be elucidated. According to previous hypothesis IgG anti-IgE autoantibodies may exert the role of specific feedback molecules that neutralize the IgE immune response induced by the cytokine network (Stadler et al. 1993a). IgG anti-IgE autoantibodies are a heterogeneous population of antibodies that execute functions of physiological control of the specific IgE Th2 immune response, as inhibition of IgE synthesis and removal of IgE from CD23 receptor (Stadler et al. 1993b). In atopic diseases as asthma these autoantibodies have been implicated in the modulation of the IgE immune response, mainly because they may react with epitopes located in C epsilon 2 domain of IgE, which is involved in the binding of this immunoglobulin on Fcε high affinity receptor (FcεRI) on the surface of basophils and mast cells, preventing the sensitization of these cells (Shakib & Powell-Richards 1991, Stadler et al. 1995).

Immunoblotting analysis carried out with material eluted from Sepharose-protein G beads used in serum IgG depletion confirmed the presence of IgG anti-IgE autoantibodies in all sera tested and evidenced significant cleavage of IgE under physiologic conditions. A frequent 75 kDa Mr band corresponding to IgE ε-heavy chain and polypeptides of different size, all carrying ε-heavy chain epitopes, were revealed with specific goat IgG anti-human IgE peroxidase conjugate in the blots carried out with sera from the three groups, including healthy control sera. Apparently, such pattern of IgE degradation may reflect the action of intrinsic regulatory mechanisms used by the organism to block the formation of circulating immune complexes with IgG anti-IgE autoantibodies, avoiding therefore inflammation and autoimmunity. The observation of an unusual fragment of IgE only in VL sera needs to be investigated. Current studies are evaluating the participation of *L. chagasi* proteases in enzymatic digestion of this immunoglobulin during infection, after binding to CD23 receptor on parasited macrophages.

The information that IgG anti-IgE autoantibodies contribute significantly for serum immunoglobulin E loss in laboratory procedures of immunoglobulin G depletion using immobilized specific ligands is a relevant subject in immunodiagnosis assays. The main consequence of this sequestration of immune complexes formed by IgG and IgE, is an accentuated fall in sensitivity of the immunoassays projected to detect specific serum IgE antibodies in vitro and consequently occurrence of false negative results. Additionally, this finding determine caution during the interpretation of IgG antibody positive tests obtained with solid phase immunoassays covered with immobilized antigens and sera presenting concomitant high concentrations of IgE specific antibodies and IgG anti-IgE autoantibodies, as observed in Th2 immune responses from allergic or parasitic diseases.

REFERENCES

- Atta AM, D'Oliveira Jr A, Correa J, Atta MLB, Almeida RP, Carvalho EM 1998. Antileishmanial IgE antibodies: a marker

- of active disease in visceral leishmaniasis. *Am J Trop Med Hyg* 59: 426-430.
- Bennich H, Ishizaka K, Ishizaka T, Johansson SG 1969. A comparative antigenic study of γE-globulin and myeloma-IgND. *J Immunol* 102: 826-831.
- Coffman RL, Varkila K, Scott P, Chatelain R 1991. Role of cytokines in the differentiation of CD4+ T cells subsets *in vivo*. *Immunol Rev* 123: 1-19.
- Elkayam O, Tamir R, Pick AI, Wyszynski A 1995. Serum IgE concentrations, disease activity and atopic disorders in systemic lupus erythematosus. *Allergy* 50: 94-96.
- Gruber BL, Kaufman LD, Marchese MJ, Roth W, Kaplan AP 1988. Anti-IgE autoantibodies in systemic lupus erythematosus. *Arthritis & Rheumatism* 31: 1000-1006.
- Huber A, Genser D, Spitzauer S, Scheiner O, Jensen-Jarolim E 1998. IgE/anti-IgE immune complexes in sera from patients with Crohn's disease do not contain food-specific IgE. *Int Arch Allergy Immunol* 115: 67-72.
- Hunter CA, Reiner SL 2000. Cytokines and T cells in host defense. *Cur Opin Immunol* 12: 413-418.
- Ingnan M, Johansson SGO, Bennich H 1981. Anti-IgE autoantibodies in human serum: occurrence and specificity. *Int Arch Allergy Appl Immunol* 65: 51-56.
- Kojima S, Yokagawa M, Tada T 1972. Raised levels of serum IgE in human helminthiasis. *Am J Trop Med Hyg* 21: 913-918.
- Mazza DS, Grieco MH, Reddy MM, Meriney D 1995. Serum IgE in patients with human immunodeficiency virus infection. *Ann Allergy Asthma Immunol* 74: 411-414.
- Millauer N, Zuercher AW, Miescher SM, Gerber HA, Seitz M, Stadler BM 1999. High IgE in rheumatoid arthritis (RA) patients is complexed with anti-IgE autoantibodies. *Clin Exp Immunol* 115: 183-188.
- Quinti I, Brozek C, Wood N, Geha RS, Leung DYM 1986. Circulating IgG autoantibodies to IgE in atopic syndromes. *J Allergy Clin Immunol* 77: 586-594.
- Romagnani S 1998. T cell subsets (Th1, Th2) and cytokines in autoimmunity. In NR Rose, IR Mackay (eds), *The Autoimmune Diseases*, Academic Press, London, p. 163-191.
- Romagnani S 2000. T-cell subsets (Th1 versus Th2). *Ann Allergy Asthma Immunol* 85: 9-18.
- Scheuer A, Haas H, Schlaak M 1991. Prevalence and subclass distribution of IgG-anti-IgE autoantibodies in atopy and parasitosis. *Int Arch Allergy Appl Immunol* 96: 271-276.
- Shakib F, Powell-Richards A 1991. Elucidation of the epitopes locations of human autoanti-IgE: recognition of two epitopes located within the C epsilon 2 and the epsilon 4 domains. *Int Arch Allergy Appl Immunol* 95: 102-108.
- Sousa-Atta MLB, Araújo MI, D'Oliveira Jr A, Ribeiro de Jesus A, Almeida RP, Atta AM, Carvalho EM 1999. Detection of specific IgE antibodies in parasite diseases. *Braz J Med Biol Res* 32: 1101-1105.
- Stadler BM, Stampfli MR, Miescher S, Furukawa K, Vogel M 1993a. Biological activities of anti-IgE antibodies. *Int Arch Allergy Immunol* 102: 121-126.
- Stadler BM, Stampfli MR, Miescher S, Rudolf M, Vogel M 1995. Cloning of human anti-IgE autoantibodies and their role in regulation of IgE synthesis. *Int Arch Allergy Immunol* 107: 48-50.
- Stadler BM, Stampfli MR, Vogel M, Aebischer I, Furukawa K, Holzner ME, Rudolf MP, Miescher S 1993b. A specific feedback by anti-IgE autoantibodies on the cytokine network in allergy. *Agents and Actions* 40 (Suppl.): 144-152.

