Acta Derm Venereol (Stockh) 1989; Suppl 144: 67–69

IgG Anti IgE in Atopic Dermatitis

I. QUINTI, R. PAGANELLI, G. MARONE[1] and F. AIUTI

Department of Allergy and Clinical Immunology, University of Rome "La Sapienza", and
[1]Department of Internal Medicine, II University of Naples, Italy

Autoantibodies specific for immunoglobulins have been demonstrated in sera of patients with various diseases, but also in healthy subjects, in particular after antigenic stimulation, with increasing incidence with age. In some diseases, particularly rheumatoid arthritis, these antiglobulins have been well characterized, and their participation in immune complex formation with serum immunoglobulins of the corresponding specificity has been confirmed. In 1971 Williams demonstrated that sera from allergic patients contained circulating IgM antibodies directed against IgE (1). Subsequently, different authors reported the occurrence of IgG autoantibody to IgE in sera from patients with asthma, atopic dermatitis, rhinitis and chronic urticaria (table 1, also for references 1–8). Atopic dermatitis (AD) is a common skin disorder characterized by several immunological abnormalities. Although the pathogenetic role of IgE in the inflammatory response of AD remains uncertain, there is universal agreement that IgE bound to basophils and mast cells, once bridged by a specific antigen, trigger the release of histamine and other mediators to initiate the inflammatory response (9). We now report evidence for the presence of anti-IgE autoantibody both free and complexed with IgE in sera from patients with AD, its possible role in the pathogenesis of allergic diseases and its interference in total and specific IgE determinations.

METHODS

To measure the IgG anti-IgE antibody we used an ELISA assay. Briefly, a 96 wells microtiter plate was coated with purified myeloma IgE (ADZ). After washes, sera were added at different dilutions. After 18 h at room temperature and three more washes, alkaline phosphatase conjugated immunosorbent purified goat anti-human IgG (Zymed) was added. After washes, the substrate was added and after 30 min of color development, the optical density (OD) was read at 405 nm using a Titertek Multiskan spectrometer. Our results were also referred to a standard curve obtained with purified IgG anti IgE to estimate the amount of IgG anti IgE of the sample. We obtained the purified IgG anti IgE from the serum of a child with atopic eczema. 3 ml of serum were passed on an immunosorbent Sepharose column (dimensions 1.2×5 cm) coated with purified IgE. Immunosorbent bound IgG anti IgE were eluted with glycine HCl buffer 0.2 M, pH 2.8 and the pH quickly readjusted by addition of 2 M NaOH. The total content of IgG of the eluted antibody was measured, and the concentration adjusted to 1 mg/ml. IgE content was checked by paper radio immunosorbent assay (PRIST) and was below 100 pg/ml. This purified IgG anti IgE was also used to assess its interference with PRIST and radioallergosorbent (RAST) tests. The effect on measurements was tested by adding tenfold dilutions of the purified preparation of this autoantibody either to sera and standard (keeping constant the final volume incubated with paper discs) or to the radiolabelled anti-IgE reagent.

RESULTS

The level of IgG anti IgE but not of IgM anti-IgE were elevated in 14 out of 18 sera (Fig. 1). Significant IgG anti-IgE activity remained in 12 sera after adsorption over pooled human IgG F(ab')2 Sepharose. The IgG anti-IgE activity appeared to be directed toward the Fc portion of IgE because the absorption of positive sera over IgE (PS)-Sepharose but not over myeloma IgG Sepharose completely removed their reactivity with IgE (ADZ) and because the autoantibody reacted against the protein backbone of the FC portion of IgE synthesized from a fragment of the cloned gene of human myeloma IgE (ND) heavy chain (6). Fractionation of sera by gel filtration revealed that the IgG anti IgE activity was present both as monomeric IgG and in IgE containing immune complexes (Fig. 2). We also probed the ability of IgG anti IgE to inhibit IgE recognition by heteroantisera to IgE used in commercially available PRIST and RAST assays. Our results indicate that IgG anti IgE decreases total IgE results by 10–90% according to its weight ratio to IgE molecules present in samples devoid of endogeneous anti-IgE. Sera with high content of specific IgE to house dust mite are little affected by anti-IgE, but RAST class 2 sera can turn to negative when purified IgG anti IgE is added.

DISCUSSION

We have previously published evidence of elevated levels of IgG anti-igE antibodies in sera of allergic patients (6). In the present study we extend our obser-

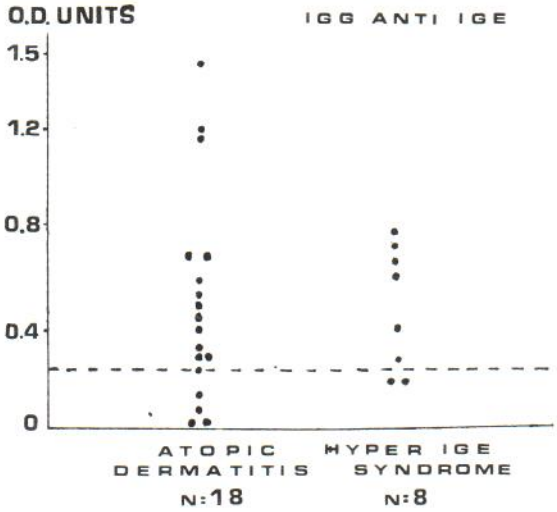

Fig. 1. IgG anti IgE (O.D.) in patients with atopic dermatitis and Hyper IgE syndrome.

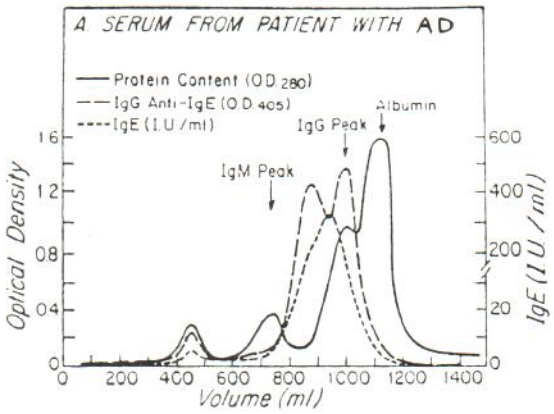

Fig. 2. Representative gel fractionation of a serum sample from a patient with atopic dermatitis. The sample was passed through a Sepharose 6B column and IgE (U.I./ml) and IgG anti IgE (O.D.) were determined in the column fractions.

vation to patients with atopic dermatitis. The epsilon chain specificity of IgG anti IgE was confirmed by competitive inhibition by its reactivity with the Fc portion synthesized from a fragment of the cloned gene of human myeloma IgE heavy chain. High titers of antibodies to isotypic determinants of IgE have been generated by immunization of mice (10) and rats (11) with syngeneic IgE. In contrast to conventional rheumatoid factors, the anti-IgE antibodies are of moderately high affinity. In rats the induction of auto anti-IgE inhibited total and specific IgE levels and had a degranulating effect on mast cells. According to our gel filtration results, we found that the anti-IgE autoantibody was present in monomeric form, but also as immune complexes with IgE. Only the monomeric form of IgG anti IgE from a patient with AD showed the capacity to trigger mast cells and basophils to release histamine in vitro (data not shown). When the same autoantibody was intradermally injected in a healthy adult an immediate wheal and flare was obtained. In five patients we could not separate the IgG anti IgE from the complexes with IgE: the complexed form was incapable of releasing histamine from basophils and PGD2 from lung mast cells (12). The dysfunction of cell mediated immunity in AD correlates with the severity of skin lesions and the levels of serum IgE. Large size immune complexes may contribute to the impairments of cell mediated immunity, chemotaxis defect and to inflammatory skin lesions associated with atopic dermatitis. Our data, moreover, demonstrate that IgG anti-IgE antibody can affect both total and specific IgE determinations: we suggest that its presence should be considered in evaluating the laboratory results.

Table I. *Summary of published studies on anti-IgE antibodies*

			(Ref.)
1972	Williams	IgM anti IgE in allergic disorders	1
1981	Inganas	IgG anti IgE in allergic asthma	2
1984	Nawata	IgG anti IgE in bronchial asthma	3
1985	Johansson	IgG anti IgE in atopic subjects	4
1985	Nawata	IgG anti IgE in atopic dermatitis	6
1986	Quinti	IgG anti IgE in atopi syndromes	7
1986	Paganelli	IgG anti IgE in Hyper IgE syndrome	8
1988	Gruber	IgG anti IgE in urticarial syndromes	9

REFERENCES

1. Williams RC, Griffiths RW, Emmons JD, Field RC. Naturally occurring human antiglobulins with specificity for E. J Clin Invest 1972; 51: 955.
2. Inganas M, Johansson SGO, Bennich H. Anti-IgE antibodies in human sera: occurrence and specificity. Int Arch Allergy Appl Immunol 1981; 65: 51.
3. Nawata Y, Koike T, Yangisawa T, Iwamamoto, Itaya T, Yoishida S, Tomioka H. Anti-IgE autoantibody in patients with bronchial asthma. Clin Exp Immunol 1984; 58: 348.
4. Johansson SGO, Magnusson CGM, Larsson B. Occurrence, specificity and putative roles of anti-IgE antibodies in human serum. 193, 1985.
5. Nawata Y, Koike T, Hosokawa H, Tomioka H, Yoshida S. Anti-IgE autoantibody in patients with atopic dermatitis. J Immunol 1985; 135: 478.
6. Quinti I, Brozek C, Wood N, Geha R, Leung DYM. Circulating IgG antibodies to IgE in atopic syndromes. J Allergy Clin Immunol 1986; 77: 586.
7. Paganelli R, Quinti I, Carbonari M, Pontesilli O, D'Offizi G, Letta T, Aiuti F. IgG anti IgE in circulating immune complexes in the hyper IgE syndrome. Clin Allergy 1986; 16: 513.
8. Gruber BL, Baeza ML, Marchese MJ, Agnello V, Kaplan A. Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. J Invest Dermatol 1988; 90: 213.
9. Rankin JA, Hitchcock M, Merrill W, Bach MK, Brashler JR, Askenase PW. IgE dependent of leukotriene C4 from alveolar macrophages. Nature 1982; 297: 329.
10. Haba S, Nisonoff A. Induction of high titers of anti-IgE by immunization of imbred mice with syngeneic IgE. Proc Natl Acad Sci USA 1987; 84: 5009.
11. Marshall JS, Bell EB. Induction of an auto-anti-IgE response in rats. I. Effects on serum IgE concentrations. Eur J Immunol 1985; 15: 272.
12. Viganò T, Sautebin L, Magni F, Crivellari NT, Paganelli R, Galli G, Berti F, Folco G. Stimulus-related differences in the formation of leukotrienes and PGD2 after immunological and non immunological challenge of human lung parenchyma in vitro. Prostaglandins, Leukotrienes and Medicine 1986; 23: 109.