

Short communication

Circulating levels of IgG1 and IgG4 anti-IgE antibodies and asthma severity

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F. Shakib¹, J. Sihoe¹, S. J. Smith¹,
P. Wilding², M. M. Clark², A. Knox²

¹ Department of Immunology and ² Respiratory Medicine Unit, Faculty of Medicine, University of Nottingham, Nottingham, UK

In this paper, we have determined the levels of IgG1 and IgG4 anti-IgE in the sera of 66 asthma patients suffering from mild ($n = 24$), moderate ($n = 23$), or severe ($n = 19$) symptoms, and 20 nonatopic, healthy subjects. The study has revealed that although asthma patients have significantly elevated levels of IgG1 and IgG4 anti-IgE antibodies, the concentration of these autoantibodies is not related to the severity of asthma. This conclusion may be related to the known heterogeneity of autoanti-IgE antibodies in terms of their ability to trigger basophil histamine release.

Key words: allergy; asthma; autoantibody; IgE; IgG1; IgG4.

Dr F. Shakib
Department of Immunology
University Hospital
Queen's Medical Centre
Nottingham NG7 2UH
UK

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Several groups of investigators have detected IgG anti-IgE antibodies in the sera of a large proportion of patients with atopic disease (reviewed in 3), particularly allergic asthma, where anti-IgE is found in more than 95% of patients (6). We have recently confirmed and extended these observations by showing that circulating levels of IgG anti-IgE antibodies which are significantly above those found in normal persons are detectable in about 60% of allergic asthma patients (9) and 90% of persons infected with hookworm (*Necator americanus*) (12).

In terms of subclass distribution, IgG anti-IgE activity is restricted to IgG1 and IgG4, with IgG4 displaying a disproportionately high percentage of the activity (3, 9), particularly in IgE:anti-IgE complexes (10). The subclass nature of IgG anti-IgE is of potential physiologic and pathologic relevance, because IgG subclasses exhibit considerable variation in their ability to trigger or bind to effector function molecules (C1q and Fc γ R).

No studies have so far been conducted to investigate the clinical relevance of IgG subclass anti-IgE antibodies in asthma patients. In this paper, we have addressed this issue by comparing the levels of IgG1 and IgG4 anti-IgE in the sera of 66 asthma patients suffering from mild ($n = 24$), moderate ($n = 23$), or severe ($n = 19$) symptoms.

Material and methods

Asthma patients

A total of 66 asthma patients (35 men and 31 women), recruited from a register of volunteers and from hospital outpatient clinics, were included in this study. Patients gave written informed consent to the study, which was approved by the Nottingham City Hospital Ethics Committee. Patients were seen on a single occasion for completion of questionnaire, skin prick tests, venesection, and lung function measurements. Most of the patients (54/66 or 82%) were atopic, as shown by a positive skin test (defined as wheal and flare reaction 3 mm greater than the negative control) to one or more of the following allergen sources (Bencard, Brentford, UK): house-dust mite (*Dermatophagoides pteronyssinus*), grass pollen, and cat fur.

Lung function measurements

FEV₁ (forced expiratory volume in 1 s) and FVC (forced vital capacity) were measured on a dry-bellows spirometer (Vitalograph®, Vitalograph Ltd, Buckingham, UK) as the higher of two readings within 100 ml. The PD₂₀ methacholine (provocative dose of methacholine causing a 20% fall in FEV₁)

was performed in patients with base-line FEV₁ > 50% predicted by the method of Yan et al. (14). After measurement of base-line FEV₁, patients inhaled three puffs of normal saline, followed by increasing doses of methacholine to give cumulative doubling doses of methacholine from 0.048 to 24.5 µmol. FEV₁ was measured 1 min after inhalation of saline and after each dose of methacholine until it had fallen by 20% from the postsaline value. PD₂₀ was calculated by linear interpolation of the last two readings on the log dose-response plot. Beta₂-agonists were withheld for > 6 h before measurement of PD₂₀. After the first visit, patients recorded twice daily PEF (peak expiratory flow rate) for 7 days as the best of three readings from a mini-Wright peak flow meter. PEF readings were expressed as absolute values and also as diurnal variability (calculated as amplitude of mean).

Assessment of asthma severity

Assessment of asthma severity was made according to the consensus report of the National Heart, Lung, and Blood Institute (USA) (5). The assessment criteria included clinical features (e.g., frequency and duration of symptoms and exacerbations), lung function measurements (FEV₁, PEF, and PD₂₀ methacholine), and type of medication required to control symptoms (β₂-agonists and inhaled or systemic corticosteroids). On the basis of these criteria, patients were divided into three groups: mild (*n* = 24, age range 23–49, mean 38), moderate (*n* = 23, age range 19–56, mean 40), and severe (*n* = 19, age range 16–62, mean 45). Serum samples were obtained from these patients at the time of clinical assessment and frozen until they were tested for IgG1 and IgG4 anti-IgE and total IgE.

Control group

Serum samples were also obtained from 20 apparently healthy, nonatopic (serum IgE range 2–84 IU/ml; mean 32 IU/ml) members of the Department of Immunology (University of Nottingham, UK). This control group consisted of eight men and 12 women aged 20–61 (mean 29) years.

IgG1 and IgG4 anti-IgE ELISA

The IgE protein used to coat the ELISA microtitre plate wells was isolated from the plasma of IgE myeloma patient WT (plasma kindly provided by Dr Denis Stanworth, Department of Immunology, University of Birmingham, UK). The IgE protein was isolated in pure form by euglobulin precipitation and two rounds of affinity chromatography on protein G Sepharose 4 fast-flow gel (to remove IgG contami-

nants) and on CNBr-activated Sepharose 4B gel coupled with rabbit antiwhole human serum (to remove albumin and other plasma proteins), respectively. The purity of IgE was assessed at each step by immunoelectrophoresis and Ouchterlony gel diffusion. Furthermore, IgE-coated plates gave negligible background values (less than 0.05 OD₄₀₅) when directly treated with mouse monoclonal antihuman IgG1 and IgG4 antibodies.

IgG1 and IgG4 anti-IgE antibody levels in serum samples from asthmatic and normal subjects were measured by a previously described (9, 12) enzyme-linked immunosorbent assay (ELISA). Briefly, the method involves incubating serum samples (1/5) in IgE-coated (2.3 µg/ml) microtitre wells and detecting bound IgG1 and IgG4 with mouse monoclonal antihuman IgG1 (NL16) and mouse monoclonal antihuman IgG4 (GB7B), respectively. The colour reaction was then developed with alkaline phosphatase conjugated goat antimouse IgG and *p*-nitrophenyl phosphate as enzyme substrate. Results were expressed in OD₄₀₅ units by taking the mean values of duplicate estimations corrected for background (i.e., serum added to wells not coated with IgE). The interassay variability of this method, which was monitored with well-defined positive and negative serum samples, is less than 10%.

Total IgE assay

Serum total IgE concentration was measured by the Milenia IgE kinetic enzyme immunometric assay (DPL Limited, Glyn Rhonwy, UK), as described previously (7). This method has been fully validated in our laboratory (R. Jones, personal communication) against the Pharmacia ImmunoCAP System (1).

Statistical analysis

The Mann-Whitney U test was used to compare mean values of IgG1 and IgG4 anti-IgE. Correlation coefficients were calculated by Spearman correlation.

Results

Levels of IgG1 and IgG4 anti-IgE antibodies and total IgE found in the sera of 24 mildly, 23 moderately, and 19 severely asthmatic subjects and 20 apparently healthy, nonatopic subjects are summarized in Table 1.

With an OD₄₀₅ value equal to or above 0.1 taken to be positive, the frequency of IgG1 and IgG4 anti-IgE activity in the sera of asthmatic subjects was in the order IgG1 (39/66; 59%) > IgG4 (31/66; 47%), with all the grossly elevated levels being found in

Table 1. Summary of IgG1 and IgG4 anti-IgE and total IgE levels measured in sera of 24 mild (Mi), 23 moderate (Mo), and 19 severe (S) asthma patients and 20 nonatopic healthy (N) subjects

Variable	Units	Group	Mean	Range	Standard error
IgG1 anti-IgE	OD ₄₀₅	Mi	0.152	0.048–0.551	0.023
		Mo	0.143	0.051–0.399	0.019
		S	0.165	0.017–0.938	0.047
		N	0.052	0.000–0.226	0.013
IgG4 anti-IgE	OD ₄₀₅	Mi	0.100	0.061–0.200	0.007
		Mo	0.121	0.051–0.253	0.012
		S	0.121	0.028–0.219	0.014
		N	0.061	0.016–0.115	0.008
Total IgE	IU/ml	Mi	162*	11–4227	199
		Mo	314*	24–2721	157
		S	76*	1–13204	688
		N	18*	2–84	16

* Geometric mean.

IgG1. In contrast to this pattern, the incidence of a positive IgG1 and IgG4 anti-IgE activity in the control group was very low: 2/20 (10%) for IgG1 and 4/20 (20%) for IgG4. The differences in the OD₄₀₅ values of IgG1 anti-IgE ($P = 0.0000$) and IgG4 anti-IgE ($P = 0.0000$) between the control group and the three asthmatic groups (each tested separately) were highly significant. However, there were no statistically significant differences in the OD₄₀₅ values of IgG1 and IgG4 anti-IgE between the mildly, moderately, and severely asthmatic groups (Table 1). Furthermore, there were no significant correlations between levels of IgG1 and IgG4 anti-IgE and results of lung function tests (i.e., FEV₁, PEF_R, and PD₂₀) in the asthmatic patients as a whole and in the subgroups.

The mean value of serum total IgE levels in the three asthmatic groups was in the order severe (821 IU/ml) > moderate (663 IU/ml) > mild (515 IU/ml), with the differences between the means of the severe and moderate ($P = 0.0004$) and the moderate and mild ($P = 0.0169$) groups being significant.

Discussion

Cross-linking of mast cell- and basophil-bound IgE molecules by allergen leads to the release of mediators of type I hypersensitivity (e.g., allergic asthma). The fact that such cross-linking and consequent release of mediators can also be effected *in vitro* by heterologous anti-IgE antibody has fuelled interest in the pathologic role of IgG autoanti-IgE antibodies, which are prevalent in asthma patients (3, 6, 9). A previous study (8), which was based on measuring whole IgG (rather than subclasses of IgG) anti-IgE, failed to show a correlation between autoanti-IgE levels and asthma severity in children. However, given the subclass heterogeneity of IgG anti-IgE in

asthma patients (3, 6, 9), we thought it would be interesting to investigate the relationship between circulating levels of IgG1 and IgG4 anti-IgE antibodies and asthma severity.

However, the present study has revealed that although asthma patients have significantly elevated levels of IgG1 and IgG4 anti-IgE antibodies, the concentration of these autoantibodies is not related to the severity of asthma as defined here. There are several possible explanations for this. For instance, our observation may be explained in terms of suppression of autoanti-IgE production as a result of treatment with systemic (in the severe group) and high doses of inhaled (in the moderate group) corticosteroids. However, we feel that failure to show a relationship between asthma severity and circulating levels of anti-IgE is more likely to be due to the known heterogeneity of autoanti-IgE antibodies in terms of their ability to trigger basophil histamine release. Thus, several investigators have shown that not all IgG autoanti-IgE preparations can activate basophils *in vitro* (2, 4). We have recently confirmed and extended these studies by showing that only a small proportion of circulating IgG anti-IgE from asthma patients can trigger *in vitro* histamine release from basophils of an asthmatic subject (4/21; 19%), a hay fever sufferer (4/10; 40%), and a healthy person (7/21; 33%), and that the ability of autoanti-IgE to release histamine is not related to its titre or IgG subclass distribution (13). Therefore, it is reasonable to assume that the histamine-releasing ability of circulating IgG autoanti-IgE is related to its epitope specificity, particularly since we have already demonstrated two epitope-specific subpopulations of IgG anti-IgE in asthma patients: C_ε2-C_ε3 inter-domain-specific (aa301-339) and C_ε4 domain-specific (11).

In conclusion, future studies of this kind should ideally be done by measuring epitope-specific IgG anti-IgE, an undertaking which may well be enhanced by the future availability in our laboratory of monoclonal phage-encoded antihuman IgE Fab fragments, which could be employed in inhibition assays for the rapid definition of the epitope specificity of a large number of sera.

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