

Circulating human IgG autoanti-IgE antibodies in asthma patients block the binding of IgE to its high affinity receptor

S J Smith, A Galvin, I Hall, F Shakib

Abstract

Aims—To investigate the ability of circulating human IgG autoanti-IgE antibodies from asthma patients to block the binding of IgE to the α chain of the high affinity receptor (Fc ϵ RI).

Methods—This involved the use of a well validated flow cytometric method to detect inhibition of FITC labelled IgE binding to a fibroblast cell line (CHK1E1) transfected with the α chain of Fc ϵ RI.

Results—IgG autoanti-IgE-containing sera blocked the binding of IgE-FITC to the CHK1E1 cells. No such inhibition was demonstrable with rheumatoid sera containing autoanti-IgG (that is, rheumatoid factor) but lacking autoanti-IgE. Percentage inhibition (up to 50%) of IgE binding to the CHK1E1 cells was directly related to the titre of IgG1, but not IgG4, autoanti-IgE in the sera tested (correlation coefficient 0.66, probability 0.003).

Conclusions—The capacity of anti-IgE to block the binding of IgE to Fc ϵ RI has important clinical implications, particularly in terms of downregulation of allergic reactions.

(J Clin Pathol: Mol Pathol 1995;48:M148-M152)

Keywords: Asthma, autoanti-IgE, high affinity receptor, IgE.

IgG autoanti-IgE is detectable in the serum of individuals with enhanced IgE production, namely those with allergic conditions such as asthma,¹⁻⁴ and with parasitic infestations.⁵ Our investigations of the subclass profile of IgG autoanti-IgE have revealed that most of the activity is found in IgG1 and IgG4, with IgG4 displaying a disproportionately high proportion of the activity, particularly in IgE:anti-IgE complexes.⁶ We have shown previously that the autoantibody response in asthma patients is directed against two epitopes located within the C ϵ 2-C ϵ 3 interdomain region (aa301-339) and the C ϵ 4 domain of IgE respectively.⁷ Significantly, the high affinity receptor binding site on IgE (aa330-335)⁸ is contained within one of the sequences (aa301-339) recognised by IgG autoanti-IgE. Thus an autoantibody of such epitope specificity would potentially block the binding of IgE to mast cells, thereby down-regulating the allergic release of mediators. Experimental evidence in support of this notion has recently been provided by Hellman⁹ who showed that the induction of an autoanti-IgE

response in rats, by immunisation with a recombinant C ϵ 2-C ϵ 3 fragment, suppressed the binding of IgE to its high affinity receptor on mast cells.

The high affinity receptor for IgE (Fc ϵ RI) is a tetrameric structure consisting of one α , one β , and two disulphide bonded γ polypeptide chains. Only the α chain is responsible for IgE binding.⁸ The β chain is involved in signal transduction and the γ chains facilitate correct expression and orientation of the receptor. Fc ϵ RI is predominantly found on mast cells and basophils, but recent reports suggest that it may also be present on eosinophils,¹⁰ monocytes,¹¹ and Langerhans cells.¹²

The recent availability of a fibroblast cell line transfected with the α and γ subunits of human Fc ϵ RI¹¹ provides a direct system for studying the ability of human autoanti-IgE to modulate the binding of IgE to Fc ϵ RI. In this study we show the ability of IgG anti-IgE-containing sera, obtained from asthma patients, to block the binding of IgE to such fibroblasts.

Methods

ANTIBODY REAGENTS

Mouse monoclonal anti-human IgE antibodies were obtained from Dr P E Brenchly, Department of Immunology, St Mary's Hospital, Manchester, UK (clones E1 and E3) and Professor B M Stadler, Institute of Clinical Immunology, Bern, Switzerland (clones BSW17 and Le27). The plasma of an IgE myeloma patient (WT) and purified myeloma IgG protein were provided kindly by Dr D R Stanworth (Department of Immunology, University of Birmingham, UK). The mouse anti-human IgG monoclonal antibodies [anti-IgG1 (clone NL-16), anti-IgG2 (clone GOM1), anti-IgG3 (clone ZG4) and anti-IgG4 (clone GB7B)] and the alkaline phosphatase conjugated goat anti-mouse IgG were purchased from Oxoid-Unipath. The recombinant human IgE-Fc domains were gratefully provided by Dr B Sutton (Molecular Biology and Biophysics Group, The Randall Institute, King's College, London, UK). The single IgE domains were expressed in *E Coli* and refolded; the IgE-Fc fragment (C ϵ 2-C ϵ 4) was expressed in NSO cells and was glycosylated as described elsewhere.¹³

PURIFICATION OF HUMAN IgE

IgE was isolated from plasma by euglobulin precipitation and affinity chromatography. The purity of the IgE preparation was assessed by

University of Nottingham Medical School, Nottingham: Division of Molecular and Clinical Immunology
S J Smith
A Galvin
F Shakib

Department of Therapeutics
I Hall

Correspondence to: Dr F Shakib, Division of Molecular and Clinical Immunology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH, UK.

Accepted for publication 28 February 1995

immuno-electrophoresis, and its concentration determined by a Milenia IgE kinetic EIA (DPC).

FLUORESCIN ISOTHIOCYANATE LABELLING OF IgE AND IgG

One ml of the IgE or IgG protein (2.5 mg/ml) was incubated for three hours at 37°C with fluorescein isothiocyanate (FITC) isomer 1 on 10% Celite (Calbiochem) in 0.15 M sodium chloride containing 0.1 M sodium hydrogen carbonate. The samples were passed through a Sephadex G-25 column (Pharmacia) to remove unbound FITC. Fractions containing FITC labelled IgE or IgG were collected and pooled and the FITC to protein ratio was determined by measuring optical density at 280 nm and 495 nm.

IGG SUBCLASS ANTI-IgE MEASUREMENT

IgG1 anti-IgE and IgG4 anti-IgE antibodies were measured in the sera of asthmatic individuals using an enzyme linked immunosorbent assay (ELISA) described previously. Briefly, the procedure involved incubating a 1/5 dilution of the serum samples in IgE or IgE-Fc coated microtitre plates and detecting bound IgG1 and IgG4 anti-IgE with mouse monoclonal anti-human IgG1 and IgG4, followed by alkaline phosphatase conjugated goat anti-mouse IgG. Results were expressed in OD405 units by taking the mean value of duplicate estimations, corrected for non-specific binding (that is, samples added to wells not coated with IgE).

SERUM SAMPLES

Blood samples were obtained, with informed consent, from allergic asthma patients attending the asthma outpatient clinic at University Hospital, Queen's Medical Centre, Nottingham. Samples which were found to be positive for IgG anti-IgE were used in the IgE blocking experiments (see below). Sera from rheumatoid arthritis patients containing auto-antibodies to IgG (that is, rheumatoid factor) were used as controls. Such rheumatoid factor positive sera did not have detectable IgG anti-IgE.

CELL CULTURES

The method used for constructing the fibroblast cell line transfected with the α and γ subunits of the human FcεRI (CHK1E1) and the original untransfected cell line (CHO) is described elsewhere.¹¹ CHO was cultured in Iscove's modified Dulbecco medium (IMDM) containing 10% heat inactivated fetal calf serum (FCS) (Seralab, JRH Biosciences) and 100 U/ml penicillin + 100 µg/ml streptomycin. CHK1E1 cells were cultured in IMDM, 10% FCS, and 1 mg/ml gentamicin (Gibco, Life Technologies). Both cell lines were cultured to confluence and split every three days. PBS containing 0.05% trypsin/0.02% EDTA (Gibco, Life Technologies) was used to detach

the cells from the bottom of the culture flask. Cells were washed in PBS containing 1% bovine serum albumin (BSA) and then re-suspended to a concentration of 10⁶ per ml.

EXPRESSION OF FcεRI α CHAIN ON THE CHK1E1 CELLS

A 100 µl volume of 10 µg/ml FITC labelled IgE or IgG was incubated for 15 minutes at room temperature with 100 µl of either CHK1E1 or CHO cells (10⁶ cells per ml) and 100 µl of PBS containing 0.1% BSA, and then washed with 2 ml of PBS containing 0.1% azide (to prevent capping). Unlabelled IgE was used to assess autofluorescence. The cells were re-suspended and fixed in 0.3 ml Sysmex cellpack containing 0.5% formaldehyde. They were collected on a FACScan (Becton Dickinson) with a linear fluorescence setting of 660 volts. The fluorescence (FL1) profile versus forward scatter (FSC) was used to monitor the cells, and the amplification scale was altered according to the level of fluorescence. For each sample, 4000 events were collected by the FACScan and then analysed by means of the flowMATE program (DAKO).

DEMONSTRATION OF THE IgE BINDING SPECIFICITY OF THE CHK1E1 CELLS

A 50 µl volume of 20 µg/ml (that is, 5.32×10^{-8} M) FITC labelled IgE (patient WT) was incubated for 15 minutes at room temperature with 50 µl of $10^{-6.4} - 1.33 \times 10^{-8}$ M of either recombinant human ε constant region domains (Cε2-Cε4, Cε2, Cε3) or unlabelled human IgE before incubation with 100 µl of CHK1E1 cells (10⁶ cells per ml) and 100 µl of PBS containing 0.1% BSA. The cells were subsequently prepared for FACScan analysis as described previously.

To ascertain the IgE binding specificity of CHK1E1 cells, blocking experiments were performed whereby 100 µl volume of 10 µg/ml FITC labelled IgE (WT) was incubated for one hour at 37°C with 100 µl of a range of anti-human IgE monoclonal antibodies (E1, E3, BSW17, and Le27) and four anti-human IgG subclass monoclonal antibodies (NL16, GOM1, ZG4, and GB7B) as controls. Each mixture was then incubated for 15 minutes at room temperature with 100 µl of CHK1E1 cells (10⁶ cells per ml). The cells were subsequently prepared for FACScan analysis as described previously.

THE USE OF IgG ANTI-IgE CONTAINING SERA TO BLOCK IgE-FITC UPTAKE BY CHK1E1 CELLS

All serum samples were incubated for 30 minutes at 56°C to dissociate IgE from anti-IgE. (The IgE site responsible for binding to anti-IgE and the α chain of FcεRI is heat labile.) Thus complexed IgG anti-IgE was rendered available for ligand interaction. To investigate percentage inhibition at different concentrations of IgE-FITC, we used a serum sample (patient FC) that was shown in the initial screen to block the binding of IgE-FITC to the

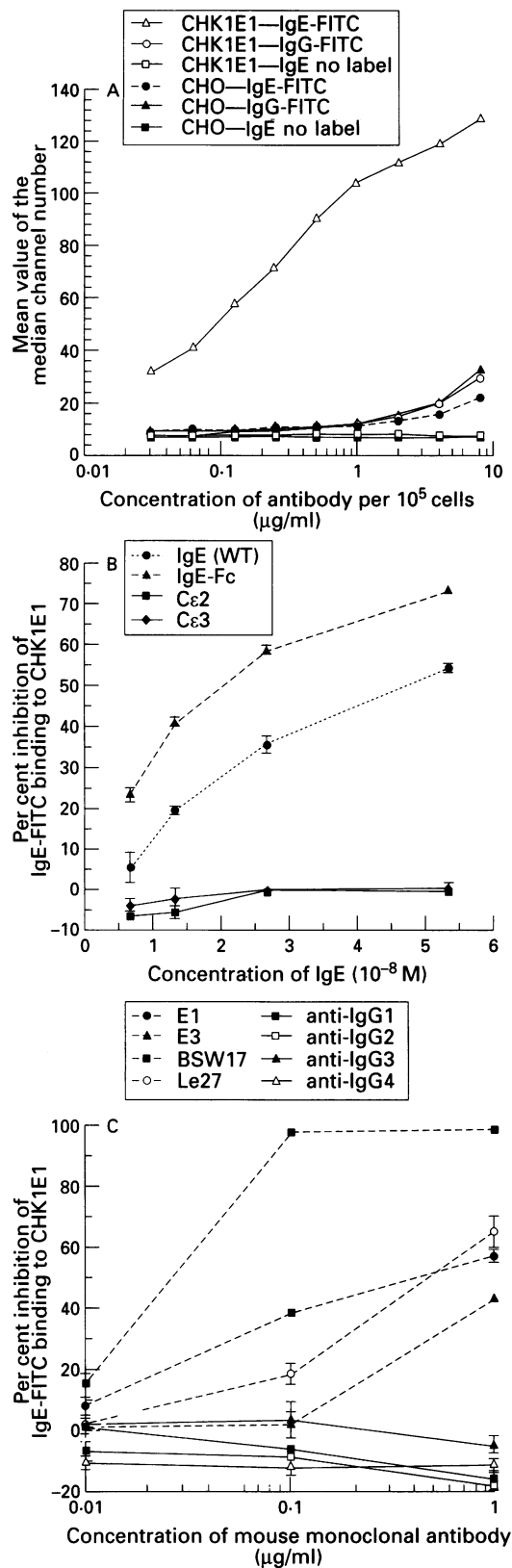


Figure 1 The IgE binding specificity of the CHK1E1 cell line. (A) The sigmoidal dose-response curve shows the uptake of IgE-fluorescein isothiocyanate (FITC) by CHK1E1 cells. No such binding was demonstrable with the non-transfected CHO cells. As expected, neither cell line showed significant fluorescence when incubated with either unlabelled IgE or IgG-FITC. (B) Dose dependent inhibition of binding of IgE-FITC to the CHK1E1 cells using unlabelled IgE and a recombinant IgE-Fc (Cε2-Cε4). No such inhibition was demonstrable with recombinant Cε2 and Cε3 fragments. (C) Dose dependent inhibition of binding of IgE-FITC to the CHK1E1 cells using mouse anti-human IgE monoclonal antibodies E1, E3, BSW17, and Le27. No such inhibition was demonstrable with mouse anti-human IgG subclass monoclonal antibodies.

CHK1E1 cells. Thus 100 μl of serum was incubated for one hour at 37°C with 100 μl of IgE-FITC (0.2–1.0 μg/ml), and the mixture was then incubated for 15 minutes at room temperature with 100 μl of CHK1E1 cells (10⁶ cells per ml). Finally, the cells were prepared for FACS analysis as described earlier. In subsequent blocking assays IgE-FITC was used at a concentration of 0.25 μg/ml.

PURIFICATION OF IgG AUTOANTI-IgE

A euglobulin fraction was prepared from the serum of an asthmatic patient (FC), with high titre of IgG anti-IgE, by precipitation with 33% saturated solution of ammonium sulphate. The IgG fraction was isolated on protein G-sepharose and IgG anti-IgE was subsequently isolated from the IgG fraction by affinity chromatography on IgE-sepharose. The protein content of the IgG anti-IgE antibody obtained in this way was quantitated using nephelometry and the preparation tested for anti-IgE activity by ELISA.

STATISTICAL ANALYSIS

Correlation coefficients between IgG subclass anti-IgE titres and the ability to inhibit the binding of FITC labelled IgE to the CHK1E1 cells was calculated using Spearman correlation.

Results

THE IgE BINDING SPECIFICITY OF CHK1E1 CELLS
A dose-response curve was produced from a plot of the median channel number against the concentration of IgE-FITC per 10⁵ CHK1E1 cells. The binding curve of IgE-FITC to the CHK1E1 cells was sigmoidal (fig 1A). IgG-FITC and unlabelled IgE did not cause any significant increase in the median channel number when assayed at concentrations less than 1 μg/ml per 10⁵ CHK1E1 cells. There was no significant binding of IgE-FITC, unlabelled IgE, or IgG-FITC to the untransfected cell line at antibody concentrations below 1 μg/ml per 10⁵ CHO cells.

The specificity of the interaction of IgE with the α chain of the CHK1E1 cells was further ascertained in a competitive assay using purified human IgE and recombinant ε constant region domains. The binding of IgE-FITC to the CHK1E1 cells was inhibited in a dose dependent manner by unlabelled human IgE (patient WT) and by a recombinant fragment spanning the Fc portion of IgE (that is, Cε2–Cε4) (fig 1B). However, the binding of IgE to the CHK1E1 cells was not affected by adding the same molar concentrations of recombinant Cε2 or Cε3 domains. Furthermore, the binding of IgE-FITC to the CHK1E1 cells was inhibited in a dose related fashion with the four anti-human IgE monoclonal antibodies, but not with the anti-human IgG subclass monoclonal antibodies (fig 1C).

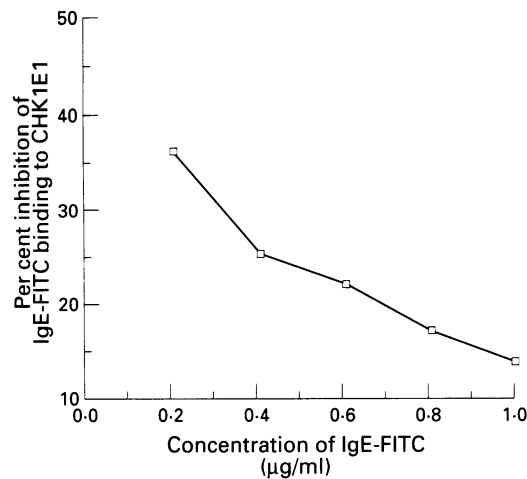


Figure 2 Percentage inhibition of IgE-fluorescein isothiocyanate (FITC) binding to CHK1E1 cells with an IgG anti-IgE containing serum sample (patient FC). The serum sample was assayed at various concentrations of IgE-FITC.

BLOCKING OF IgE-FITC BINDING TO CHK1E1 CELLS WITH SERA CONTAINING IgG ANTI-IgE

A plateau of fluorescence, as defined by negligible changes in the median channel number, was observed at concentrations of IgE-FITC above 1 µg/ml per 10^5 CHK1E1 cells (fig 1A). Therefore 1 µg/ml of IgE-FITC was taken to be the saturating concentration for the α chains on the CHK1E1 cells. Thus, in assessing the ability of IgG anti-IgE to block the binding of IgE to the CHK1E1 cells, 0.25 µg/ml of IgE-FITC was used to give 50% saturation of the α chains on 10^5 CHK1E1 cells. Percentage inhibition was also maximal at this IgE-FITC concentration (fig 2).

Twenty asthmatic patients' sera containing anti-IgE were used to investigate the effect of IgG autoanti-IgE on the binding of IgE-FITC to the α chain of CHK1E1 cells. Results show that the IgG anti-IgE-containing sera inhibited the binding of IgE-FITC to the CHK1E1 cells. This inhibition was found to be directly dependent on the titre of IgG1, but not IgG4, anti-IgE in the sera tested, as shown by a highly significant correlation coefficient (0.66, probability 0.003) (fig 3). The specificity of this blocking effect was further demonstrated by the observation that none of the 15 control (rheumatoid) sera, containing anti-IgG but lacking anti-IgE, inhibited the binding of IgE-FITC to CHK1E1.

BLOCKING OF IgE-FITC BINDING TO CHK1E1 CELLS WITH PURIFIED IgG ANTI-IgE

Affinity purification of IgG anti-IgE from the serum of an asthmatic patient (FC) showed that the IgE blocking activity seen in fig 2 is fully recoverable in the IgG fraction. However, further affinity purification of IgG anti-IgE led to a significant decrease in its IgE blocking activity, possibly because high affinity anti-IgE antibodies resisted elution from the IgE column.

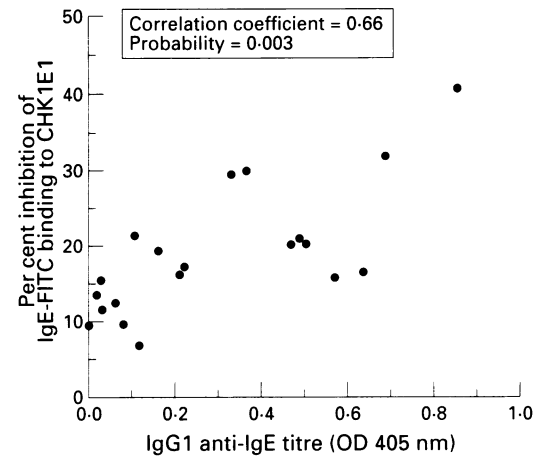


Figure 3 The relationship between serum IgG1 anti-IgE titre and per cent inhibition of IgE-fluorescein isothiocyanate (FITC) binding to the CHK1E1 cells. The plot shows a direct relationship between the two variables, which was statistically significant (correlation coefficient 0.66, $p=0.003$).

Discussion

We have shown previously that the IgG anti-IgE autoantibody response in asthma patients is directed predominantly against the Cε2-Cε3 interdomain region.⁷ Significantly, the high affinity receptor (FcεRI) binding site on IgE (aa330-335)⁸ is contained within the sequence (aa301-339) recognised by IgG autoanti-IgE thereby suggesting that an autoantibody of such epitope specificity would potentially block the binding of IgE to its high affinity receptor. To explore this possibility, we have used a fibroblast cell line (CHK1E1) transfected with the α and γ subunits of the human FcεRI. The advantage of using the transfected cell line approach (as opposed to mast cells, basophils, and eosinophils) for this purpose is its greater simplicity, because there is no requirement to dissociate endogenously bound IgE from the cells when assessing the binding of IgE to FcεRI. Also, human mast cells, basophils, and eosinophils are not readily obtainable in pure form.

We initially demonstrated the IgE binding specificity of the transfected cell line. In addition, we have shown that the binding of IgE to the receptor occurs through the Fc portion of IgE, as indicated by the competitive inhibition experiment using recombinant human IgE-Fc. As expected, such inhibition was not demonstrable with recombinant Cε2 or Cε3 domains because, although the FcεRI binding site is located within the Cε3 domain (aa330-335), the structural integrity of the entire Fc region is apparently required for receptor engagement.⁸ The IgE binding specificity of the CHK1E1 cells was further ascertained by showing that a range of mouse monoclonal anti-human IgE, but not anti-human IgG, was able to inhibit this interaction.

Having established the validity of our experimental system, we tested 20 serum samples, taken from asthma patients with positive titres of IgG1 or IgG4 anti-IgE or both, for their ability to block the binding of IgE-FITC to the

CHK1E1 cells. This revealed inhibition of up to 50%. Such inhibition correlated directly with the titre of circulating IgG1 anti-IgE but not IgG4 anti-IgE. The blocking of IgE binding to the CHK1E1 cells by serum was related to its IgG fraction. However, further affinity purification of IgG anti-IgE led to a significant decrease in its IgE blocking activity, possibly because high affinity anti-IgE antibodies resisted elution from the IgE column. The recently reported IgG autoanti-FcεRI α chain antibodies in sera of urticaria patients could also potentially block the binding of IgE to the CHK1E1 cells.¹⁴ However, we have excluded the possibility that such autoantibodies are present in the sera of asthma patients (data not shown). We have also shown that the IgG anti-IgE antibodies we have been measuring are not anti-idiotypes, as they were equally reactive with IgE and IgE-Fc.

None of the 15 rheumatoid sera containing anti-IgG but lacking anti-IgE that we tested was able to cause inhibition of IgE binding to the CHK1E1 cells. Although the IgG anti-IgE-containing asthmatic patients' sera caused modest inhibition (up to 50%) of IgE binding to the CHK1E1 cells, we believe that the experimental conditions used in this study underestimated the IgE blocking effect of anti-IgE *in vivo*. It is estimated that human basophils and mast cells have about 10⁵ FcεRI molecules per cell and in non-atopic individuals only a small percentage (3–4%) of the receptors is occupied by IgE.¹⁵ Percentage saturation is unlikely to be much higher in asthma patients who show moderate increases in circulating levels of IgE compared with, for instance, atopic eczema patients. It is therefore important to note that the inhibition values presented in this paper were obtained using IgE-FITC to α chain ratio (giving 50% saturation of receptors) at least 10-fold higher than those found *in vivo*. We are also likely to have used a much higher IgE to anti-IgE ratio than is the case in the human circulation.¹⁶

The demonstration that human autoanti-IgE blocks the binding of IgE to its high affinity receptor *in vitro* is very much in accord with the recent work of Hellman,⁹ who showed that the induction of an autoanti-IgE response in rats, by immunisation with a recombinant Cε2–Cε3 fragment, abrogated *in vivo* mast cell activation. We have also shown previously that autoanti-IgE antibodies isolated from asthma patients modulate allergen induced basophil histamine release.¹⁷ Taken together, these data

suggest that the stimulation of autoanti-IgE production, particularly those directed against the Cε2–Cε3 interdomain region,⁷ has therapeutic potential in allergic individuals. The great attraction of this strategy is that therapy is aimed at stimulating the production of a naturally occurring, rather than a novel, antibody.

The authors would like to thank Dr J P Kinet and Dr M H Jouvin for their generous gift of the fibroblast cell lines and Dr A R Robins for advice on using the FACScan. SJS is supported by a grant from the Wellcome Trust (036661/2/92/Z) and FS is a recipient of a British Lung Foundation/Midlands Electricity Board Research Fellowship. IH is a National Asthma Campaign Senior Lecturer.

- Nawata Y, Koike T, Yanagisawa T, Iwamoto I, Itaya T, Yoshida S, *et al*. Anti-IgE autoantibody in patients with bronchial asthma. *Clin Exp Immunol* 1984;58:348–56.
- Koike T, Tsutsumi A, Nawata Y, Tomioka H, Yoshida S. Prevalence and role of IgG anti-IgE antibody in allergic disorders. *Monogr Allergy* 1989;26:165–75.
- Shakib F, Boulstridge L, Smith SJ. A study of the inter-relationship between circulating IgG subclass anti-IgE autoantibodies, IgE and soluble CD23 in asthma. *Allergol Immunopathol* 1993;21:20–4.
- Shakib F, Sihoe J, Smith SJ, Wilding P, Clark MM, Knox A. Circulating levels of IgG1 and IgG4 anti-IgE antibodies and asthma severity. *Allergy* 1994;49:192–5.
- Shakib F, Pritchard DI, Walsh EA, Smith SJ, Powell-Richards A, Kumar S, *et al*. The detection of autoantibodies to IgE in plasma of individuals infected with hookworm (*Necator americanus*) and the demonstration of a pre-dominant IgG1 anti-IgE autoantibody response. *Parasite Immunol* 1993;15:47–53.
- Shakib F, Mills CS, Powell-Richards A. The recognition of a recombinant human Fcε fragment by the subclasses of IgG anti-IgE: disproportional subclass distribution of complexed autoantibody. *Int Arch Allergy Immunol* 1992; 97:243–8.
- Shakib F, Powell-Richards A. Elucidation of the epitope locations of human autoanti-IgE: recognition of two epitopes located within the Cε2 and the Cε4 domains. *Int Arch Allergy Appl Immunol* 1991;95:102–8.
- Sutton BJ, Gould HJ. The human IgE network. *Nature* 1993;366:421–8.
- Hellman L. Profound reduction in allergen sensitivity following treatment with a novel allergy vaccine. *Eur J Immunol* 1994;24:415–20.
- Gounni AS, Lamkhoulou B, Ochiai K, Tanaka Y, Delaporte E, Capron A, *et al*. High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* 1994;367:183–6.
- Maurer D, Fiebiger E, Reininger B, Wolffwiniski B, Jouvin MH, Kilgus O, *et al*. Expression of functional high affinity immunoglobulin E receptors (FcεRI) on monocytes of atopic individuals. *J Exp Med* 1994;179:745–50.
- Bieber T. FcεRI on human Langerhans cells: a receptor in search of new functions. *Immunol Today* 1994;15:252–3.
- Young RJ, Owens RJ, Mackay GA, Chan CNW, Shi J, Hide M, *et al*. Secretion of recombinant human IgE Fc by mammalian cells and biological activity of glycosylation site mutants. *Protein Eng* (in press).
- Hide M, Francis DM, Grattan CEH, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993;328:1599–604.
- Ishizaka T, Soto C, Ishizaka K. Mechanisms of passive sensitisation. III. Number of IgE molecules and its receptor sites on human basophil granulocytes. *J Immunol* 1973;111:500–6.
- Paganelli R, Quint I. Pathological significance of circulating IgG anti-IgE complexes. *Monogr Allergy* 1989;26:184–97.
- Shakib F, Smith SJ. *In vitro* basophil histamine-releasing activity of circulating IgG1 and IgG4 autoanti-IgE antibodies from asthma patients and the demonstration that anti-IgE modulates allergen-induced basophil activation. *Clin Exp Allergy* 1994;24:270–5.