

Studies on the association between immunoglobulin E autoreactivity and immunoglobulin E-dependent histamine-releasing factors

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SUMMARY

It has been reported that serum immunoglobulin E (IgE) from certain atopic patients can sensitize basophils to release histamine in response to IgE-dependent histamine-releasing factors (HRFs). It has also been shown that patients suffering from severe forms of atopy may contain IgE autoantibodies. It was investigated whether HRF-responsive sera contained IgE autoantibodies and if there was an association between IgE autoreactivity and IgE-dependent responsiveness to HRF. The presence of HRF-responsive IgE (IgE⁺) in serum of patients with respiratory atopy was determined by stimulating stripped human basophils sensitized by serum with peripheral blood mononuclear cell (PBMC)-derived HRF, and measuring the release of histamine. In parallel, these sera were screened for the presence of IgE autoantibodies to nitrocellulose-blotted human cellular extracts. The capacity of IgE autoantigen-containing preparations to induce histamine release was tested in the stripped basophil assay. Eleven out of 52 sera contained IgE autoantibodies to blotted cellular extracts of human PBMCs or of the human epithelial cell line A431. No significant association was found between IgE autoreactivity and IgE-dependent responsiveness to HRF: 7/26 IgE⁺ sera contained IgE to human cellular extracts, and 4/26 of the sera without IgE⁺ did also. IgE autoantigen-containing extracts did not induce histamine release of appropriately sensitized basophils. By size-exclusion chromatography it was shown that a 32 000 MW autoantigen eluted in the >55 000 MW fraction, which indicates that this protein forms polymers or complexes with other macromolecules. This might explain the discrepancy between binding and histamine-releasing activity. A 20 000 MW IgE-defined autoantigen cross-reacted with a shrimp allergen. Our results indicate that IgE-reactivity to immunoblotted human protein and IgE-dependent HRF activity are distinct entities that may co-occur in atopic patients.

INTRODUCTION

Acute allergic symptoms are induced by the crosslinking of immunoglobulin E (IgE) antibodies on the surface of an effector cell (e.g. mast cell, basophil) by an allergen, which results in degranulation of mediators such as histamine.¹ It is also well established that basophils can be activated to release mediators by IgE-independent histamine-releasing factors (HRF).² Evidence for the presence of another type of HRF came from studies indicating that culture supernatants of human cells contained IgE-dependent HRF.^{3,4} IgE-dependent HRF, by definition, require the presence of

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Abbreviations: AD, atopic dermatitis; PBMC, peripheral blood mononuclear cells; HRF, histamine-releasing factor; HRF_{mn}, HRF produced by mononuclear cells; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SAS, saturated ammonium sulphate.

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IgE to induce histamine release and only certain types of IgE, designated IgE⁺, exert this reactivity.³ By definition, sera that fail to sensitize basophils for responsiveness to HRF were termed IgE⁻. Our group investigated the IgE-dependent histamine-releasing activity in culture supernatants of stimulated human peripheral blood mononuclear cells (PBMCs) using the stripped basophil bioassay.^{4,5} In this assay IgE antibodies are removed from human basophils with an acidic buffer, the cells are re-sensitized by serum, and the histamine release is investigated in response to stimuli. IgE-dependent responsiveness to HRF produced by mononuclear cells (HRF_{mn}) was shown to be associated with atopic sensitization.⁵ HRF_{mn}-responsive IgE was present in 40% of the sera from allergic rhinitis and asthma patients whereas it was absent in non-atopics.⁵ Sampson *et al.*⁶ showed that mononuclear cells from children with atopic dermatitis (AD) and food hypersensitivity produced IgE-dependent HRF_{mn}, which declined with allergen avoidance and clinical remission of the skin disorder. Moreover, serum of these AD patients rendered basophils capable of secreting histamine in response to HRF_{mn}, whereas serum from control subjects did not.

There are several possibilities to explain the responsiveness of IgE⁺. The feature discriminating IgE⁺ from other IgE antibodies might be present in the constant regions of IgE. IgE⁺ might be a differently glycosylated IgE³ or an alternative splice variant of the IgE molecule.⁷ It is known that a combination of two partial agonists can activate basophils. Accordingly, it is possible that both HRF and a structurally different form of IgE are necessary to achieve full basophil activation. HRF might then exert a priming effect on basophils. The histamine-releasing activity of the IgE-dependent HRF p23 cloned by MacDonald *et al.*⁸ might be due to such a priming process.

Alternatively, IgE⁺ reactivity might not be mediated by IgE alone, but in combination with IgG anti-IgE antibodies.^{9,10} Anti-IgE antibodies have been described to modulate basophil histamine release in a stimulating as well as in a blocking manner.^{10,11}

A third possibility would be that IgE⁺ is a HRF-specific IgE antibody, and HRF an IgE autoantigen. HRF would then exert its reactivity, similar to conventional allergens, by cross-linking IgE on the surface of basophils. In this context it is noteworthy that several studies reported that patients suffering from severe forms of atopy contain IgE autoantibodies.¹² IgE autoantibodies have been detected in sera from atopic dermatitis (AD) patients, recognizing different proteins in human cellular extracts on immunoblot.¹³ A strong IgE binding was associated with severe forms of atopic eczema.¹⁴ Several IgE-binding human proteins have been cloned by screening cDNA expression libraries from human tissues:^{15,16} Hom s 1 (55 kDa), Hom s 2 (10 000 MW), Hom s 3 (20 000 MW), Hom s 4 (36 000 MW), and Hom s 5 (43 000 MW).¹⁴ Other IgE autoantigens have been described by the group of Crameri *et al.*^{17–19} The allergens manganese-dependent superoxide dismutase (26 000 MW), acidic ribosomal phosphoprotein type 2 (11 000 MW) and cyclophilin B (22 000 MW) from *Aspergillus fumigatus* were IgE cross-reactive with their

human homologues. It is not clear whether autoreactive IgE is produced during an allergic reaction to exogenous allergens, or, alternatively, that autoreactive IgE is induced by endogenous allergens released during the chronic allergic reaction. The fact that some autoreactive IgE antibodies are cross-reactive with an exogenous allergen indicates that autoreactive IgE production is in some instances induced by an exogenous allergen.

We have not been able to show binding of IgE to HRF_{mn} with Western blotting and immunoprecipitation experiments. However, the concentration and the purity of HRF_{mn} in our preparation might be too low for detection in these tests. In the basophil histamine release assay it is possible to detect low amounts of allergen and in the presence of a high concentration of irrelevant proteins.²⁰ From model systems with common allergen we know that the sensitivity of the basophil assay is <0.05 ng/ml. The material used for stimulation with HRF_{mn} had a total protein content of 537 µg/ml, which reflects the low purity of the HRF_{mn} preparation.

The primary question of the current study was whether two published observations, IgE autoreactivity and IgE-dependent histamine-releasing activity, were related. For this purpose we screened sera from atopic patients for the presence of IgE autoantibodies to blotted proteins and analysed some IgE-defined autoantigens in more detail. In parallel, we tested sera for their capacity to sensitize stripped basophils to release histamine in response to PBMC-derived HRF. Furthermore it was studied whether IgE autoantigen-containing preparations induce histamine release in passively sensitized basophils. Our finding that IgE autoreactivity and IgE-dependent HRF activity seem to be distinct entities is discussed.

MATERIALS AND METHODS

Preparation of human cellular extracts

Cellular extracts of the human cervix carcinoma cell line HeLa S3, and the monocyte-like cell lines U937 and MonoMac 6 were prepared according to the method described in the protocol for cytoplasmic extracts by Verheijden *et al.*²¹ Preparing extracts from the human epithelial cell line A431 and peripheral blood mononuclear cells was performed as described previously.^{13,14}

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting

SDS–PAGE, blotting to nitrocellulose and IgE immunodetection of extracts from A431 and PBMCs were performed as described previously¹⁴ and of HeLa, MonoMac, and U937 extracts as described by Roberts *et al.*²² Extracts from A431 and PBMCs were run on a reduced 12.5% polyacrylamide gel (0.2 mg protein/cm), the other extracts on a reduced 15% gel (0.5 mg protein/cm). In short, sera were added to the nitrocellulose strips 1:10 diluted in buffer containing 0.5% bovine serum albumin (BSA). In order to mimic the *in vivo* situation as closely as possible, the amount of serum was not adjusted for the total IgE. After overnight incubation, immunodetection was performed

following similar incubation with ^{125}I -labelled anti-IgE. To visualize IgE binding, the blots were exposed to X-ray film (Kodak, New York, NY) at -70° for 1 week. In the case-control study serum from patients with AD as classified according to Hanifin and Rajka²³ were used as positive control, because AD sera are reported to contain IgE autoantibodies.¹² Serum from AD patients D4, D5, D8, D11, MD, S, and M contained a total IgE of 4668, 6951, 9070, 1251, 1800, 2600, and 11 000 IU/ml, respectively, and were radioallergosorbent test (RAST) positive to at least one inhalant allergen. Since it has been described that *Aspergillus* allergens might cross-react with human proteins¹⁷ plasma #141, #144, and #145 which are RAST positive to *Aspergillus* (>15 IU specific IgE/ml) were also tested. IgE binding of serum from patients with respiratory atopy was compared to that of a serum of a non-atopic subject (serum VR, total IgE: 19 IU/ml, RAST negative to common inhalant allergens). Bands present in the negative control were excluded from analysis. The scoring of the blots was performed independently by two persons. IgE binding was classified into three categories: positive, negative and doubtful. Serum with a clear IgE binding to one or more human proteins was scored as positive. Only serum judged as positive by both was termed IgE autoreactive serum; serum with a doubtful or no IgE binding negative serum.

Stripped basophil histamine release bioassay

The histamine release assay was performed as described previously.^{24,25} In short, after removal of IgE from surface of basophils by an acidic buffer, the cells were sensitized by 150 μl human serum for 90 min at 37° . Stimulations (37° , 60 min) were performed in 1 mM CaCl_2 using 2.5×10^6 cells in 250 μl and 50 μl allergen or 100 μl cellular extract or HRF preparation. To up-regulate IgE-mediated histamine release, the stimuli were diluted in HEPES buffer/1 mM CaCl_2 containing recombinant interleukin-3 (IL-3, final concentration 600 pM; Pepro Tech Inc., Rocky Hill, NJ). Histamine was determined by fluorometric analysis as described by Siraganian.²⁶

Production of IgE-dependent HRF_{mn}

IgE-dependent HRFs produced by human PBMC were prepared as described elsewhere.²⁷ In short, PBMC obtained by leucapheresis were isolated by Percoll centrifugation and elution, and stimulated for 18 hr with streptokinase/streptodornase (SK/SD) (Lederle Laboratories, Pearl River, NY). IgE-independent HRFs were removed from the culture supernatant by heparin Sepharose CL-6B (Pharmacia Biotech, Uppsala, Sweden). Eleven HRF-preparations from different donors selected out of 29 preparations on basis of high reactivity were pooled and further purified by size-exclusion chromatography (Sephadex G75). Five fractions containing IgE-dependent histamine-releasing activity were pooled, and 10 times concentrated. The concentration of protein was 9.4 mg/ml. A culture medium preparation was produced in the same way for control experiments.

Calculation of HRF-responsive IgE levels

Since the histamine release experiments were performed on different days, an adjustment had to be made for interassay variability. The histamine release of basophils sensitized by 150 μl of serum was determined upon challenge with different concentrations of HRF_{mn} (threefold dilutions, final protein concentration: 20–537 $\mu\text{g/ml}$). The HRF-response of the patients was related to the HRF dose-response curve of reference plasma #151. Plasma #151 was allotted 100 sensitizing units/ml, which corresponded to a histamine release of $66 \pm 9\%$ ($n=8$, mean $\pm$ SD) to 537 $\mu\text{g/ml}$ of HRF_{mn} preparation. A serum containing more than three sensitizing units/ml was defined as HRF_{mn}-reactive serum ($>15\%$ histamine release to 537 $\mu\text{g/ml}$ of HRF_{mn} preparation).

Characterization of atopic patients

This study was performed after written informed consent of the patients and was approved by the local ethical committee.

Twenty-six IgE⁺ and 26 IgE[−] sera from atopic non-asthmatics and asthmatics were described before²⁸ (Table 1). These sera were tested in the case-control analysis to determine the correlation between IgE auto-reactivity to cellular extracts of human cell lines and IgE-dependent responsiveness to HRF. Moreover, an additional group of atopic non-asthmatic or mild asthmatic patients (15 IgE⁺ and 19 IgE[−]) and non-atopics ($n=15$) were tested for IgE binding to extracts of the HeLa cell line. These latter sera were not included in the case-control analysis.

Besides sera described above, five plasma samples from the diagnostic panel of the CLB were tested (Table 2). Plasma was defibrinated by recalcification and dialysed. The plasma samples were used to study the specificity and cross-reactivity of the IgE binding in more detail.

Analysis of IgE-reactive autoantigens

Ammonium sulphate fractionation. HeLa cellular proteins were precipitated with ammonium sulphate (30%, 50%, 100% saturation) at 4° . In the 30%, 50% and 100% saturated ammonium sulphate (SAS) precipitates, respectively, 22%, 21%, and 14% of the total protein was present.

Size exclusion chromatography. One ml of 100% SAS precipitate (10.7 mg protein) was fractionated by size-exclusion chromatography using Sephadex G75 (35×1.2 cm). After elution of 14 ml (35% of column volume), fractions of 3 ml were collected. Fraction A contained the bulk of the protein (molecular weight $>55\,000$ MW); fractions B (25 000–55 000 MW) and C (10 000–25 000 MW) were the next fractions. The protein concentration of the fractions was 0.94 mg/ml, 0.36 mg/ml, and 0.19 mg/ml, respectively.

Removal of specific IgE from sera. To 300 mg CNBr-activated Sepharose 4B (Pharmacia) 12 mg shrimp protein, or 6 mg chironomid protein was coupled. Allergen-specific IgE antibodies were removed from 500 μl serum by absorption with an equal volume of allergen Sepharose (50 mg Sepharose/ml) for 4 h while rotating. Removal of

Table 1. Characteristics of patients with respiratory atopy

Patient no.	Total IgE (IU/ml)	Symptoms	IgE-dependent HRA (units/ml)	IgE autoreactivity		
				A431	PBMC	HeLa
IgE ⁺ sera						
B2	98	ANA	67	—	—	—
R3	230	ANA	58	—	—	—
B3	206	ANA	32	+	—	—
R4	1151	ANA	28	—	—	—
R5	250	ANA	27	—	—	—
R6	323	ANA	27	—	—	—
R7	128	ANA	24	—	—	—
R8	69	ANA	18	—	—	—
R9	58	ANA	14	—	—	—
R10	285	ANA	13	—	—	—
R11	79	ANA	4	—	—	—
A10	260	AMA	40	—	+	—
A11	507	AMA	39	—	—	—
A12	3568	AMA	28	+	+	—
A13	716	AMA	26	+	+	—
A15	189	AMA	18	—	—	—
A16	365	AMA	13	—	—	—
A17	63	AMA	12	—	—	—
A19	52	AMA	10	—	—	—
A20	178	AMA	9	—	—	—
A21	311	AMA	9	—	—	—
A23	349	AMA	8	—	+	—
A24	247	AMA	6	—	—	—
SA4	916	ASA	115	+	ND	ND
SA7	293	ASA	14	—	ND	ND
SA8	1800	ASA	7	+	ND	ND
IgE [−] sera						
B4	450	ANA	3	+	+	—
B5	42	ANA	3	—	—	—
B6	183	ANA	3	—	—	—
B7	44	ANA	3	—	—	—
R9	58	ANA	3	—	—	—
R12	86	ANA	3	—	—	—
R13	291	ANA	3	—	—	—
R14	68	ANA	3	—	—	—
R15	27	ANA	3	—	—	—
R16	108	ANA	3	—	—	—
R17	31	ANA	3	—	—	—
R19	79	ANA	3	—	—	—
R20	171	ANA	3	+	—	+
R23	39	ANA	3	—	—	—
R24	23	ANA	3	—	—	—
R25	483	ANA	3	—	—	—
A28	331	AMA	3	—	+	—
A30	264	AMA	3	—	—	—
A31	370	AMA	3	—	—	—
A32	571	AMA	3	—	—	—
A36	37	AMA	3	—	—	—
A37	96	AMA	3	—	—	—
A41	1197	AMA	3	—	+	—
A44	163	AMA	3	—	—	—
A47	418	AMA	3	—	—	—
SA11	58	ASA	3	—	ND	ND

ND, not done; ANA, atopic non-asthma; AMA, atopic mild/moderate asthma; ASA, atopic severe asthma; HRA, histamine-releasing activity.
 + serum with IgE binding to blotted proteins.

— serum with doubtful or no IgE binding to blotted proteins.

Table 2. Reference plasma samples

Patient no.	IgE-dependent HRA	IgE autoreactivity			Total IgE (IU/ml)	RAST (IU/ml)				
		HeLa	MonoMAc	U937		M	CH	C	S	T
#13	+	+	+	+	850	0.2	<0.1	2	<0.1	<0.1
#164	—	+	—	+	360	66	24	23	>100	33
#36	+	+	+	+	9100	40	ND	ND	ND	ND
#193	+	+	—	+	360	4	ND	ND	ND	ND
#151	+	+	—	+	1600	24	ND	ND	ND	ND

ND, not done; HRA, histamine-releasing activity; M, house dust mite; CH, chironomids; C, cod; S, shrimp; T, shrimp tropomyosin.

allergen-specific IgE was confirmed by RAST performed as described elsewhere.^{29,30}

RESULTS

Lack of association between IgE autoreactivity and HRF-reactivity

As positive control for the study to IgE autoreactivity, IgE binding of serum from AD patients was analyzed. In all four AD patients (one IgE[−] and three IgE⁺ sera), IgE binding to cellular extracts of the cell line A431 as well as of PBMCs was found. This result is in agreement with data published elsewhere.¹² IgE autoantibodies in sera of three atopic patients with aspergillosis were also studied. Plasma #145 bound to a protein in the A431 extract, and plasma #144 bound to a protein in the PBMC extract. No binding to an antigen with a molecular weight similar to *Aspergillus* allergens reported to be cross-reacting with human proteins was found.¹⁷

To investigate a possible association of IgE-dependent responsiveness to HRF and IgE autoreactivity, 26 IgE⁺ and 26 IgE[−] sera were tested for the presence of IgE autoantibodies (Table 1). Seven of the 26 IgE⁺ sera and 4 of the 26 IgE[−] sera contained immunoblot-detectable IgE autoantibodies to human cellular extracts. IgE-reactivity of sera to nitrocellulose-blotted A431 and PBMC cell extracts are shown in Fig. 1a and b, respectively. IgE antibodies in two IgE[−] and five IgE⁺ sera (weakly) bound to human protein in cellular extracts from the epithelial cell line A431. IgE binding to PBMC extracts was stronger than to A431 extracts. It was found in three IgE[−] and four IgE⁺ sera. Three sera (one IgE[−] and two IgE⁺ sera) recognized proteins in both extracts. The lack of a significant association between IgE autoreactivity and HRF-reactivity is summarized in Fig. 2. Of the 12 IgE⁺ sera with >20 units HRF-IgE/ml, five contained blot-detectable IgE autoantibodies while seven sera did not. Four IgE[−] sera contained IgE autoantibodies while 22 IgE[−] sera did not show IgE autoreactivity (Fig. 2).

Twenty-five IgE[−] and 23 IgE⁺ sera were also tested for reactivity to cellular extracts of the cervix carcinoma cell line HeLa (Table 1). Only serum R20 had autoantibodies to this cellular extract; a 38 000 MW protein was recognized. In an additional group of atopic non-asthmatic or mild asthmatic

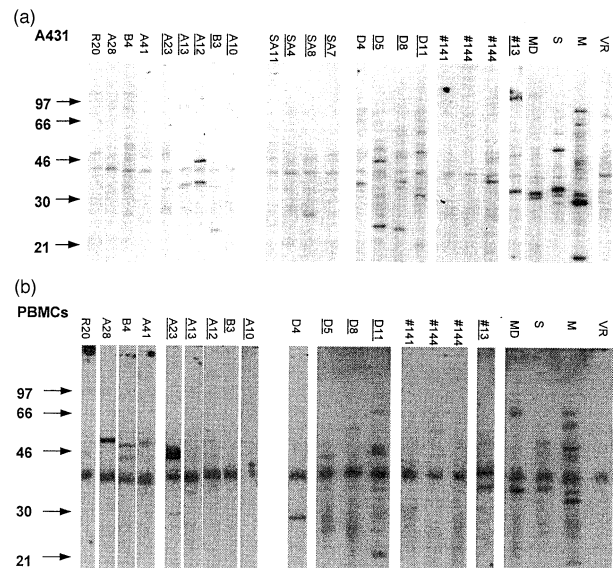


Figure 1. Detection of IgE autoantibodies in sera of atopic patients. Extracts from the (a) epithelial cell line A431 and (b) human PBMCs. Sera tested: atopic non-asthmatics and mild asthmatics ($n=48$), only the nine sera with autoreactive IgE are presented; severe asthmatic patients (SA); atopic AD patients (d); patients with aspergillosis (#141, #144, #145); plasma sample #13; positive controls MD, S, M (AD); negative control VR. A 40 000 MW component non-specifically binds radioactive anti-IgE. Underlined lane numbers: IgE⁺ serum. MD, S, M, and VR were not tested for IgE⁺ reactivity.

patients (15 IgE⁺ and 19 IgE[−] sera), one IgE[−] serum was found binding a protein of 33 000 MW. No binding was found with sera from 15 non-atopic subjects.

Analysis of HeLa-derived IgE autoantigens

IgE binding to HeLa extracts was studied in more detail using plasma samples from atopic subjects (Table 2). In Fig. 3 IgE binding of five plasma samples is shown. Both a IgE⁺ plasma (#13) and a IgE[−] plasma (#164) bound strongly to a 32 000 MW protein. In addition, IgE antibodies in plasma #164 also bound a smaller protein (20 000 MW). IgE autoreactivity was also detected to

extracts of the monocyte cell lines U937 and MonoMac (Fig. 3). Plasma #13 recognized a 32 000 MW protein also in extracts of these cell lines.

In Fig. 4a it is shown that plasma #13 also recognized the protein under non-reducing conditions (apparent MW of 41 000). Size-exclusion chromatography of the 100% SAS fraction of HeLa extract might indicate that the 32 000 MW protein is in complex with other protein(s). IgE binding of plasma #13 was strongest to the high molecular weight fraction (> 55 000 MW) whereas this protein migrated at 32 000 MW on SDS-polyacrylamide gel (Fig. 4b).

Because the IgE antibodies present in plasma samples #13 and #164 bound to an autoantigen of the same molecular weight, these samples were studied in more detail. Because both plasma samples happened to be RAST positive to seafood extracts (shrimp and/or cod) (Table 2), it was studied whether the 32 000 MW protein on immunoblot was human tropomyosin^{31,32} an important cross-sensitizing allergen. IgE against shrimp or chironomid were

removed from the plasma samples after which IgE binding to HeLa proteins was studied. IgE from the absorbed plasma samples still bound to the 32 000 MW protein; however, the binding to the 20 000 MW protein of plasma #164 was abolished by absorption with shrimp extract (Fig. 5). Absorption with chironomid extract did not change IgE binding. HeLa cells thus contain a 20 000 MW IgE autoantigen that cross-reacts with a shrimp allergen.

Preparations containing native IgE autoantigens do not induce histamine release

To determine whether the IgE binding proteins in extracts of human cells had histamine-releasing activity, the stripped basophil assay was used. Plasma samples with IgE autoantibodies (Fig. 3) were tested. Despite strong IgE-reactivity to HeLa cell-derived autoantigens on immunoblot, the histamine released by basophils sensitized by plasma samples #13, or #164 in response to cell extracts of HeLa (3 mg/ml) was below 5%. Even in the presence of the priming cytokine IL-3, no histamine release was induced. In another stripped basophil experiment, no histamine release was found with basophils sensitized either by plasma samples #13, or #151 in response to HeLa (3.5 mg/ml), U937 (1.5 mg/ml), or MonoMac (1 mg/ml) extracts. Plasma samples #193 and #36 were both not responsive to HeLa (3.1 mg/ml) or U937 (1.9 mg/ml) extracts. The same cellular extracts were used in the immunoblot experiments as in the histamine release experiments.

The results were remarkable because the IgE binding to human cellular extracts of especially plasma samples #13 and #164, was very strong on immunoblot. The presence of a factor inhibiting the histamine release in cellular extracts was excluded by stimulating basophils sensitized by #13 with grass pollen (1.3 µg/ml) in the presence of HeLa extract (3 mg/ml). The grass-pollen-induced-histamine release of basophils sensitized by #13 was not changed by cellular proteins: 47% and 48%, respectively, in the presence and absence of human cell extract.

The association between IgE binding and histamine-releasing activity was also studied in serum from atopic subjects without asthma or with mild asthma. As described

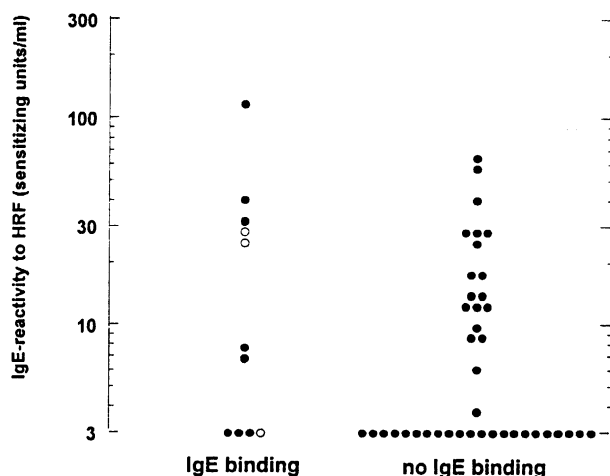


Figure 2. Lack of association between HRF_{mn}-reactivity and IgE autoreactivity. IgE binding on immunoblot was determined to extracts of the human epithelial cell line A431 and PBMCs. Open circle: serum with IgE binding to both extracts.

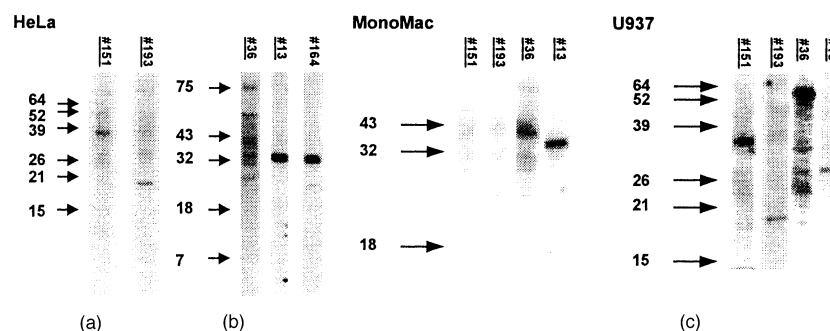


Figure 3. IgE immunoblot of extracts from (a) the cervix carcinoma cell line HeLa, and the monocyte-like human cell lines (b) MonoMac and (c) U937. Plasma samples from atopic patients were analysed for IgE binding. Underlined lane numbers: IgE⁺ serum.

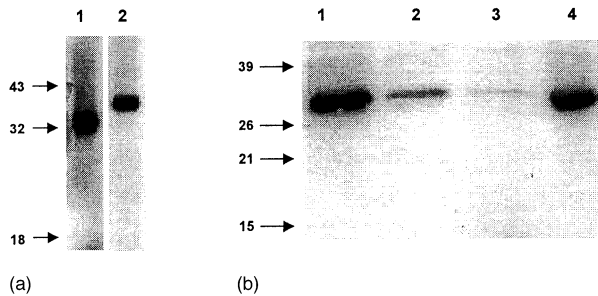


Figure 4. Analysis of a HeLa cell-derived IgE autoantigen bound by plasma #13. (a) IgE binding on immunoblot of plasma #13 to extract reduced by dithiothreitol (lane 1) and under non-reducing conditions (lane 2). (b) IgE immunoblot of fractions obtained by size exclusion chromatography of the 50–100% SAS precipitate of extracts of HeLa. Proteins present in the extract precipitating at a saturation of 50–100% ammonium sulphate were fractionated. Pool A (> 55 000 MW) (lane 1); pool B (25–55 000 MW) (lane 2); pool C (10–25 000 MW) (lane 3), HeLa 100% SAS precipitate (lane 4).

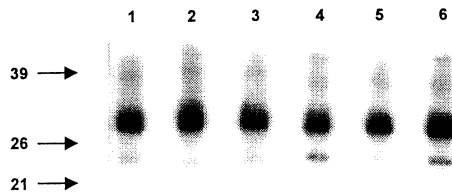


Figure 5. Cross-reactivity between a human protein and a shrimp allergen. IgE binding on immunoblot to HeLa extract by plasma samples before and after absorption was compared. Plasma #13 and #164 not absorbed (lane 1,4), absorbed Sepharose-coupled shrimp extract (lane 2,5), or by Sepharose-coupled chironomid extract (lane 3,6).

above, 2 of the 82 sera contained IgE autoantibodies to HeLa cellular extracts. Histamine release of basophils sensitized by these sera in response to extract of HeLa (3 mg/ml) was below 5%.

DISCUSSION

The main aim investigated in the present study was whether IgE autoreactivity and reactivity to IgE-dependent HRF were related phenomena. IgE-dependent responsiveness to HRF has been defined as the ability of certain atopic sera to sensitize stripped basophils to release histamine when exposed to culture supernatants of human cells. This activity depends on special features of the IgE antibodies in such sera and accordingly these sera were termed IgE⁺.³ In view of studies reporting that sera from atopic patients contain IgE autoantibodies^{13,14,33} we considered the possibility that IgE⁺ sera may contain autoreactive IgE antibodies that are directed to cell-derived autoantigens. In this scenario IgE⁺ would be autoreactive IgE and HRF an

IgE autoantigen, which might be released into the culture medium by secretion or cell death. By receptor cross-linking of IgE⁺ by HRF on the surface of basophils, histamine release might be induced, such as is the case for exogenous allergens. To address this hypothesis, HRF-reactivity of serum was determined in the stripped basophil assay using culture supernatant of human peripheral mononuclear cells as stimulus. On the other hand we determined the presence of IgE autoantibodies in atopic sera by IgE immunoblotting, using a variety of human cell extracts (HeLa cells, epithelial cells, mononuclear cells) in order to cover a broad spectrum of IgE autoantigens. However, we found no significant association between IgE⁺ and IgE autoantibodies. Seven out of the 26 IgE⁺ sera contained IgE antibodies, and 4 out of 26 IgE⁻ sera exhibited IgE autoreactivity. Even if sera with doubtful IgE binding were scored 'positive', no association was found (seven IgE⁺ and two IgE⁻ with 'doubtful IgE binding' to PBMC or A431 extract). These results suggested that IgE autoreactivity and IgE-dependent HRF reactivity are unrelated phenomena.

In a group of atopic patients, five plasma samples with IgE binding to cellular extracts of the human cervix carcinoma cell line HeLa and/or monocyte-like cell lines were studied in more detail. The assumption that IgE autoantibodies to blotted proteins are not IgE⁺ antibodies was further supported by the observation that both an IgE⁺ plasma (#13) and a IgE⁻ (plasma #164) bound very strongly to a HeLa derived autoantigen of the same molecular weight (32 000 MW). This protein was shown to be expressed in all cell types investigated in this study. The similarity between these two plasma samples was that they both contained IgE antibodies to fish and/or shrimp. We therefore were interested if the 32 000 MW IgE-defined autoantigen could be related to the major cross-reactive shrimp allergen tropomyosin.³⁴ While we found no cross-reactivity of the 32 000 MW allergen with a shrimp allergen, a 20 000 MW autoantigen cross-reacted with shrimp extract. These results support observations of Valenta *et al.*¹² and Mayer *et al.*¹⁷ that sensitization by an exogenous allergen may lead to IgE autoreactivity.

Another argument that IgE autoreactivity and IgE-responsiveness to HRF are distinct phenomena is that human cellular proteins failed to induce histamine release from basophils sensitized by five plasma samples or two sera from patients with respiratory atopy containing Western blot-detectable IgE. Despite IgE binding on immunoblot, no histamine-releasing activity was found in cellular extracts from HeLa, MonoMac, or U937. Even in the presence of IL-3, a cytokine which enhances IgE-dependent histamine release,^{35–37} basophils sensitized by plasma #13 failed to release histamine in response to cellular proteins. There are several possibilities to explain the discrepancy between IgE autoreactivity on immunoblot and the absence of histamine-releasing activity. First, the absence of histamine-releasing activity might be caused by a histamine-release inhibitory factor. However, HeLa cellular proteins did not influence histamine release to grass-pollen-extract *Dactylis glomerata*. The second possibility is that the IgE-binding sites for autoreactive IgE are exposed only after denaturation and/or

reduction on immunoblot. However, the 32 000 MW protein was also recognized by plasma #13 under non-reducing condition on immunoblot. By size exclusion chromatography it was shown that this protein eluted in the >55 000 MW fraction, which might indicate that this protein forms polymers or complexes with other macromolecules, possibly explaining the discrepancy. Whether these putative complexes are physiological, or formed during the preparation of the extract, is not known. The IgE autoantibodies might also be directed to cross-reactive carbohydrate determinants, which have been described to have a poor biological activity.³⁸ Finally, it is possible that some autoantigens identified on immunoblot are monovalent, and thus fail to crosslink IgE on the surface of basophils.

The lack of association between IgE autoantibodies and IgE⁺ might be caused by the fact that HRF_{mn} is not an IgE autoantigen, but rather acts like a priming factor on basophils. This is the case for recombinant IgE-dependent HRF p23,⁸ which does not bind IgE.^{39,40} However, we recently found that the reactivity to HRF_{mn} is not identical to recombinant HRF p23.⁴¹ Assuming that HRF_{mn} is an IgE autoantigen, the lack of association between IgE binding on immunoblot and IgE-responsiveness to HRF might be caused by the use of different allergen sources: cellular extracts in case of blotted proteins, culture supernatant of stimulated cells in case of HRF. Moreover, by conformational changes of the IgE autoantigens caused by dissociation, denaturation and reduction, the epitope recognition by IgE might be suppressed or enhanced on immunoblot. Previous results indicated that reducing conditions increase exposure of epitopes for IgE.⁴² Alternatively, it is possible that the cells express different profiles and/or amounts of IgE autoantigens depending on the culture conditions. Most likely, the concentration of HRF in the cellular extracts is low compared to total protein, particularly as the cells used for the preparation of the extracts were not stimulated.

In conclusion, no association was found between IgE-responsiveness to HRF_{mn} and IgE autoantibodies to blotted human proteins. Moreover, the lack of certain cellular IgE autoantigens to activate basophils to release histamine is in striking contrast to the definition of IgE-dependent HRF. Our study indicated that IgE autoreactivity on Western blot and IgE-dependent responsiveness to HRF_{mn} are distinct entities that may coexist in atopic sera.

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