

Immunoglobulin E Response to Human Proteins in Atopic Patients

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The demonstration that human IgE recognizes both exogenous allergens and structurally related human proteins has led to the hypothesis that IgE autoreactivity may be a pathogenic factor in atopic diseases. To determine the frequency of occurrence as well as the disease specificity of this phenomenon, we tested sera from patients with atopic diseases and, for control purposes, from persons with immunologically mediated disorders for serum IgE reactivity with nitrocellulose-blotted human proteins. We found that 12 of 20 sera from atopic patients with pronounced skin lesions contained Western blot-detectable IgE antibodies. Patients suffering predominantly

from allergic rhinoconjunctivitis as well as control individuals failed to display serum IgE autoreactivity, but occasionally exhibited elevated serum IgE levels. The molecular weights of the IgE-defined autoantigens ranged predominantly from 10 to 100 kDa. Whereas some of these were expressed in only certain cell types, others were detected in histogenetically different cells. Our results suggest that IgE autoimmunity occurs frequently in atopic dermatitis patients and may be of pathogenic relevance for the chronicity of skin manifestations typical of this disease. **Key words:** atopy/IgE autoreactivity/atopic dermatitis/Western blot. *J Invest Dermatol* 107:203–208, 1996

Atopic disorders were first described by Coca and Cooke (1923), and the hypersensitivity phenomena occurring in these diseases include IgE-mediated type I reactions (allergic rhinoconjunctivitis, allergic asthma) as well as cell-mediated type IV-like reactions (atopic dermatitis) (Coombs and Gell, 1975). Environmental substances play a causative role in many patients with respiratory forms of atopy. In the majority, these substances are water-soluble, low-molecular-weight proteins and represent either aeroallergens (pollens, mites, hair dander, fungi) or food allergens.

The immunologic mechanisms in intrinsic variants of atopy are less well understood. It is known that certain patients with dermatitis and asthma have highly elevated levels of circulating IgE antibodies, partly directed against exogenous allergens, but do not necessarily experience exacerbations of the diseases when they encounter these allergens (Juhlin *et al*, 1969; Hanifin *et al*, 1986; Sampson, 1990; Leung *et al*, 1993; Charlesworth, 1994; Cooper, 1994). In fact, some of these patients may suffer from clinical symptoms without being exposed to these allergens. Together with reports describing human dander as a potential source of anaphylactic skin reactions in atopic dermatitis patients (Hampton and Cooke, 1941; Simon, 1944; Simon, 1947; Parish, 1960; Berrens,

1970), these observations gave rise to the idea that endogenous antigens could function as triggers of atopic diseases. Further support for this concept came from the recent discovery of structural and functional similarities between environmental allergens, e.g., plant profilins (Valenta *et al*, 1991, 1993; Giehl *et al*, 1994; Staiger *et al*, 1994) and albumins from animal hair dander (Spitzauer *et al*, 1994), and human proteins. In the case of profilins, we have identified IgE antibodies in human sera reacting with both human and plant specificities (Valenta *et al*, 1991).

In the current study, we investigated whether serum IgE antibodies react with human proteins other than profilins and, if so, whether the occurrence of such IgE autoantibodies may be linked to certain disease states. For this purpose, we tested, by Western blotting, sera from atopic individuals, from patients with various immunologically mediated diseases, as well as from healthy nonallergic individuals for IgE and, for comparison, IgG reactivity against proteins from a human epithelial cell line, endothelial cells, fibroblasts, platelets, and peripheral blood mononuclear cells (PBMC) derived from different donors.

MATERIALS AND METHODS

Characterization of Patients and Controls We investigated sera from patients with atopy, chronic idiopathic urticaria, systemic lupus erythematosus (SLE), graft versus host disease (GVHD), and bullous pemphigoid, as well as from nonallergic healthy subjects. Demographic data as well as clinical and laboratory features of these patients are summarized in **Table I**. Atopic dermatitis patients with or without signs of mucosal atopy are referred to as AD patients (Hanifin and Rajka, 1980). Patients suffering from rhinoconjunctivitis with or without asthma but not from dermatitis were termed RC patients. Atopic individuals were defined by a case history indicative of type I allergy or atopic dermatitis as well as by the presence of

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Abbreviations: AD, atopic dermatitis; PBMC, peripheral blood mononuclear cells; RC, rhinoconjunctivitis; SLE, systemic lupus erythematosus.

Table I. Characterization of Atopic Dermatitis Patients and Control Groups

Group	Total IgE (kU/l)	Clinical Symptoms ^a	Age (y)	Sex	Therapy ^b	IgE Against Exogenous Allergens ^c	CAP-FEIA Absorbance ^d	IgE Against Human Proteins ^e
Atopic dermatitis								
1	2,300	d r a c	41	m	c	t g		+
2	3,800	d a	55	f	c	ap		+
3	360	d	26	m	0	t g		+
4	2,700		51	m	c	g		+
5	20	d	52	f	c	0		-
6	7,500	d	19	m	c ah	nk		+
7	8,500	d r	28	f	c ab	t g w m ap		+
8	5,100	d	17	f	c	nk		+
9	80	d	23	f	c	0		-
10	10,000	d r c a	33	m	c ah	t g m ap f		+
11	100	d r c	21	f	ah	g w m		-
12	230	d r c a	33	f	c ah	t g w m ap		-
13	400	d r c a	43	f	c ah	t g w m ap		+
14	120	d	25	m	c	nk		+/-
15	100	d	28	f	c ah	0		-
16	750	d	31	f	0	g w ap f		-
17	5,500	d r c	45	m	c ah	nk		+
18	1,800	d r c	51	m	c ah	g ap f		+
19	700	d r c	33	f	c ah	nk		+/-
20	4,800	d	50	f	c	g		+
Chronic urticaria								
1	14	u	47	f	ah			-
2	5	u	35	m	ah			-
3	14	u	49	f	ah			-
4	16	u	64	f	0			-
5	94	u	25	f	ah			-
Nonallergic healthy individuals								
1	30	0	31	m	0	0		-
2	20	0	29	f	0	0		-
Allergic rhinoconjunctivitis								
1	160	r c	31	m	ah	g t		-
2	300	r c	34	m	0	g t w		-
3	32	r c	31	m	ah	g		-
4	50	r c	25	f	0	g t		-
5	600	r c a	25	m	ah	g t		-/+
6	30	r c	31	m	0	g		-
7	80	r c	37	m	0	w f		-
Systemic lupus erythematosus								
1	20		26	f	c		555	-
2	63		25	f	c		684	-
3	1,519		35	f	c		2,019	-
4	>2,000		43	f	c		820	-
5	7		39	f	c		364	-
6	8		18	f	c		335	-
7	521		20	f	c		770	-
8	30		60	f	c		241	-
9	20		27	f	c		375	-
10	9		28	f	c		363	-
Graft versus host disease								
1	56		23	f	c, is		3,518	-
2	23		41	m	c, is		403	-
3	33		25	m	c, is		1,158	-
4	21		19	m	c, is		447	-

^a a, asthma bronchiale; c, conjunctivitis; d, dermatitis; r, rhinitis; u, urticaria.^b ab, antibiotics; ah, antihistamines; c, corticosteroids; is, immunosuppressive treatment.^c 0, no allergy; ap, allergy against animal proteins (e.g., dog, cat); f, fungal allergy; g, grass pollen allergy; m, mite allergy; nk, not known; t, tree pollen allergy; w, weed pollen allergy.^d Instead of IgE against a specific exogenous allergen source, CAP-FEIA levels for frequent allergen sources (rye grass, rye, birch, mugwort, plantain, cat, fungi-*Cladosporium herbarum*, *Alternaria alternata*, and mites) were determined. Absorbance values greater than 500 indicate the presence of specific IgE.^e +, positive with human proteins from most cell extracts (fibroblasts, epithelial cells, platelets, and endothelial cells); +/-, weak reactivity; -, no reactivity with blotted human proteins.

IgE antibodies reactive with environmental allergens, as assessed by serology and skin prick test. Elevated levels of total serum IgE were not considered a prerequisite for classifying a particular person as atopic.

Cell Lines and Protein Extracts Human umbilical vein endothelial cells were cultured as described previously (Jaffe *et al.*, 1973). Lung fibroblasts were obtained from lung cancer patients undergoing lobectomy (informed consent was given) as described (Valent *et al.*, 1989).

The human epithelial cell line A431 derived from an epidermoid mammary carcinoma was purchased from American Type Culture Collection (Rockville, MD). Platelet concentrates were obtained from the local blood bank, and PBMC were prepared from different nonallergic healthy volunteers by Ficoll density gradient centrifugation. With the exception of platelets and PBMC, all cells were cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 50 μ M β -mercaptoethanol, 100 U penicillin per ml, 100 μ g streptomycin per ml (Gibco, Gaithersburg, MD).

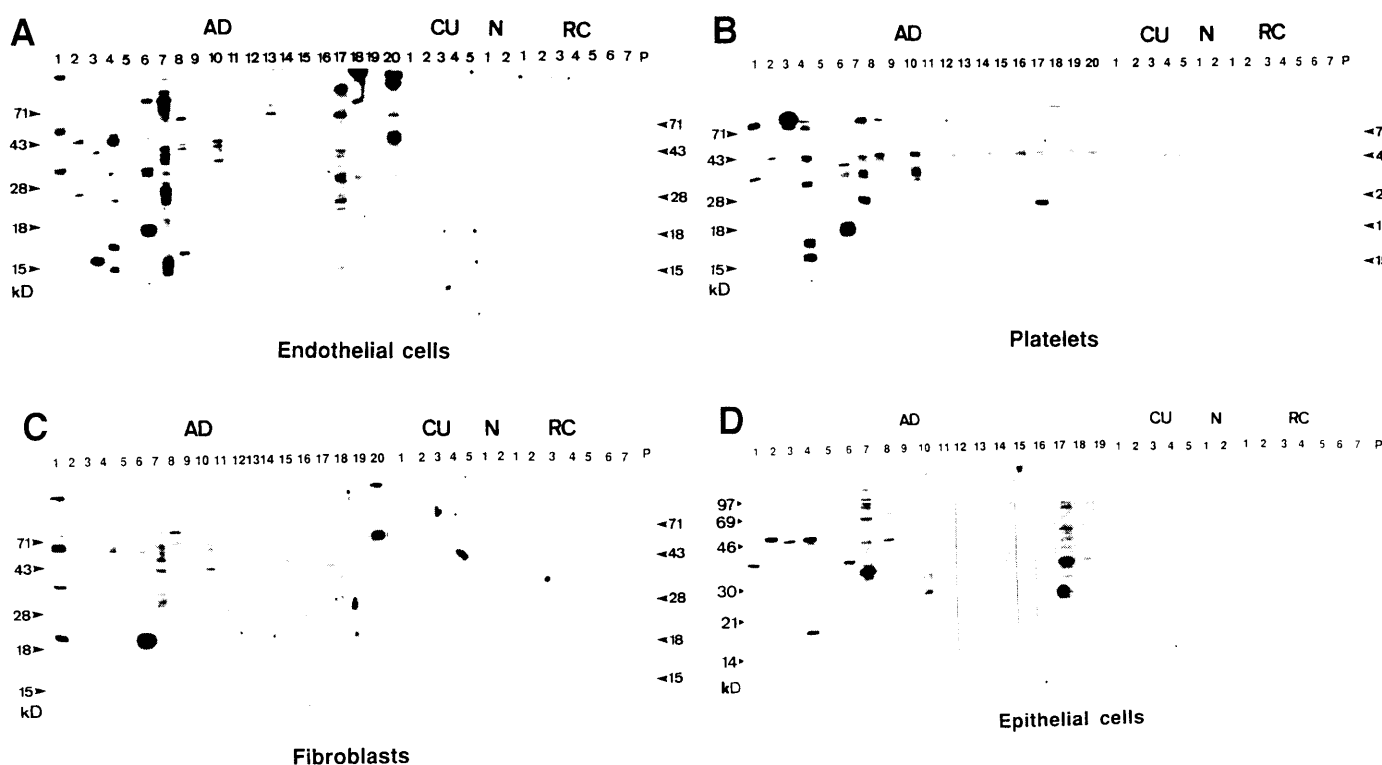


Figure 1. Atopic dermatitis (AD) patients frequently exhibit serum IgE antibodies directed against human proteins. Nitrocellulose-blotted protein extracts from human endothelial cells (A), platelets (B), fibroblasts (C), and epithelial cells (A431) (D) were probed with serum IgE from patients with AD, chronic urticaria (CU), atopic patients without dermatitis (RC), nonatopic individuals (N), and end buffer (P). Bound IgE was detected with ^{125} I-labeled anti-IgE antibodies (Pharmacia).

and 10% fetal bovine serum (FBS) (Sera Lab, Crawley Down, UK). Cells were washed twice in phosphate-buffered saline to remove medium components, and cell pellets were stored at -70°C until homogenization in sodium dodecyl sulfate (SDS)-lysis buffer (Laemmli, 1970).

Determination of Total Serum IgE Levels Blood was collected by venipuncture, and sera were stored at -20°C until analysis. The same serum samples were used for measuring total IgE levels (RIACT, Pharmacia, Uppsala, Sweden) and for immunoblotting. For the former purpose, sera were diluted 1:10 and 1:100 according to the manufacturer's protocol and assayed in duplicate. A standard curve was obtained using the standards of the RIACT kit. Values shown in Table I are given in kU/l IgE and represent mean values of duplicate determinations.

Characterization of IgE and IgG Autoantigens by Immunoblotting Cellular pellets containing approximately 5×10^6 cells each and, for control purposes, 100 μl culture medium containing 10% FBS were exposed to 1 ml SDS sample buffer, boiled for 10 min, and subjected to denaturing 12.5% preparative SDS-polyacrylamide gel electrophoresis (20 cm width) (Laemmli, 1970). Proteins were then blotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) (Towbin *et al*, 1979). Membranes were cut into strips and were incubated twice for 5 min and once for 30 min in buffer A: 50 mM sodium phosphate buffer, pH 7.4, containing 0.5% bovine serum albumin, 0.5% Tween 20, and 0.05% NaN_3 . Strips were then incubated overnight with patients' sera (sera were diluted in this buffer at 1:10 for IgE detection and 1:1000 for IgG detection) at 4°C and washed. In certain experiments, 1:10 diluted sera were preabsorbed on nitrocellulose strips containing either A431 proteins or culture medium components. Bound antibodies were detected by incubating the strips with 1:10 diluted ^{125} I-labeled anti-human IgE antibodies (RAST-Pharmacia) for 14 h at room temperature or, in the case of IgG, with a 1:1000 diluted rabbit anti-human IgG antiserum (Dako, Glostrup, Denmark) for 14 h at 4°C , followed by a 2-h incubation with a 1:1000 diluted ^{125} I-labeled donkey anti-rabbit antiserum (Amersham, Buckinghamshire, UK) at room temperature. After repeated washes, strips were dried and exposed to Kodak X-OMAT S films at -70°C using intensifying screens (Kodak, Heidelberg, Germany) for 72 h (IgE) or 24 h (IgG).

RESULTS

Patients With Cutaneous Atopy Display IgE Reactivity With Several Human Proteins Present in Different Cell Types In the first series of experiments, we found that sera from 12 of 20 AD patients and one of seven RC patients contained IgE antibodies that reacted with nitrocellulose-blotted human proteins, ranging predominantly from 10 to 100 kDa in molecular weight and extracted from histogenetically different cell types (Fig 1A-D). No such IgE reactivity was detected when sera were analyzed from five patients with chronic idiopathic urticaria (Fig 1), two nonallergic healthy persons (Fig 1), or 10 SLE and four GVHD patients (Fig 2). Immunoblot analysis of an additional 35 RC patients and 10 nonallergic control persons revealed weak serum IgE binding in the case of five RC patients, but in none of the controls (data not shown).

A more detailed analysis of the AD patients' auto- or alloreactive IgE antibody profile revealed that (i) all of the 12 positive sera reacted with proteins from more than one cell type, (ii) the number of proteins identified by the individual AD sera was highest in the case of endothelial cells as compared with extracts from the other cell types, (iii) certain proteins of identical or at least comparable molecular weight were recognized by different sera (e.g., a 35-kDa protein was detected in epithelial cell extracts by sera 1, 6, and 17), and (iv) certain sera detected proteins of similar size in different cell extracts (e.g., serum 1 detected a 35-kDa protein in endothelial cells, epithelial cells, fibroblasts, and platelets) (Fig 1).

Correlation Between Total Serum IgE Levels and the Occurrence of Serum IgE Reactivity Against Human Proteins Results obtained in the AD patient group showed a good correlation between the presence of elevated serum IgE levels and of serum IgE reactivity against human proteins (Table I). The inten-

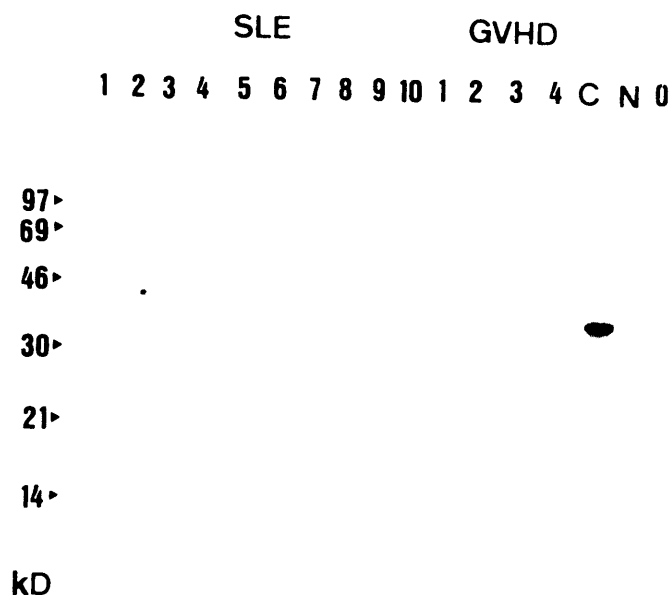


Figure 2. Patients with systemic lupus erythematosus (SLE) and graft versus host disease (GVHD) show no IgE antibodies against nitrocellulose-blotted epithelial cell (A431) proteins. Sera from patients with SLE (lanes 1–10), GVHD (lanes 1–4), atopic dermatitis (C), a nonatopic individual (N), as well as a serum-free buffer sample (0) were probed for IgE reactivity with A431 proteins. SLE and GVHD patients are characterized in Table I.

sity of IgE reactivity to nitrocellulose-blotted human proteins in the AD group, however, was not always related to the level of total serum IgE, as was exemplified by AD patient 3, who had a rather low level of total IgE (360 kU/l) but displayed pronounced IgE reactivity with nitrocellulose-blotted human proteins (see Fig 1). Our contention that hyperimmunoglobulinemia E is not an absolute prerequisite for the occurrence of IgE reactivity with human cell extracts is also supported by the observation that sera from RC patients 1 and 2 (Fig 1, Table I) as well as from SLE patients 3, 4, and 7 (Fig 2, Table I) had clearly elevated IgE levels but failed to show IgE binding to nitrocellulose-blotted human proteins.

Comparison of IgE and IgG Binding Components in Epithelial Cell Extracts To determine whether auto- or alloreactive antibodies other than IgE may occur in the sera of AD patients and perhaps other individuals, we searched for IgG antibodies against human proteins in sera from AD patients and, for comparison, from three patients with bullous pemphigoid and one nonatopic control. Figure 3 shows that only sera from AD patients exhibited IgE reactivity to several protein bands. In contrast, IgG reactivity with human proteins was observed with all but one serum sample tested. In the majority, antigenic moieties recognized by IgG differed from those identified by IgE and included prominent bands of approximately 30, 43, and 16 kDa.

Specificity of IgE Reactivity of AD Sera With Human Proteins Radioallergosorbent assay testing of the sera revealed IgE antibodies against animal proteins in certain AD sera (Table I). To exclude the possibility that IgE allo- or autoreactivities observed in this study were in part directed against animal proteins introduced in our experimental system through the use of FBS in the culture medium, we compared the IgE reactivity of certain sera with nitrocellulose strips containing blotted A431 cell extracts with those containing FBS. The immunoblots in the center of Fig 4 show the following. (i) Serum from an AD patient with animal allergy (lane 1) bound to two proteins in the molecular-weight range of albumin. The same serum detected a protein of 40 kDa in A431 cell extracts. (ii) Serum from an AD patient without animal hair or dander allergy (lane 2) reacted with a 55-kDa and a 35-kDa

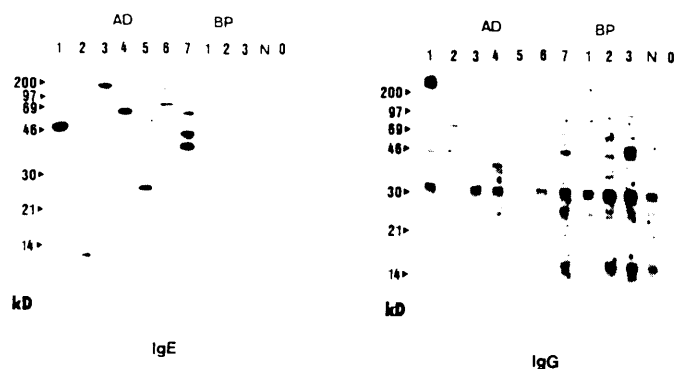


Figure 3. Comparison of IgE and IgG binding components in nitrocellulose-blotted A431 extracts. Sera from atopic dermatitis (AD) patients (lanes 1–7), patients with bullous pemphigoid (BP; lanes 1–3), a nonatopic individual (N), and buffer (0) were probed with nitrocellulose-blotted A431 proteins. Bound IgE (left) and IgG (right) antibodies were detected with ^{125} I-labeled secondary antibodies and visualized by autoradiography.

component in A431 cells extracts and showed no reactivity to medium proteins. (iii) A supernatant from the myeloma cell line U266 (Nilsson *et al*, 1970) containing more than 2000 U IgE per ml (lane 3), serum from a nonatopic individual (lane 4), or buffer (lane 5) exhibited no IgE reactivity to A431 and medium proteins. These data strongly indicate that the IgE binding components in the cell extract represent endogenous A431 proteins. This contention was further substantiated by our finding that the reactivity of AD sera with nitrocellulose-blotted A431 extracts was greatly reduced by preabsorption with A431 extracts, but not with medium (Fig 5).

A Similar Pattern of IgE-Defined Auto- or Alloantigens Is Detected in Mononuclear Cell Extracts From Genetically Unrelated Donors To investigate whether IgE reactivity with human proteins varies among different sources of donor cells, PBMC proteins were prepared from three genetically unrelated, nonallergic donors (Fig 6A–C), separated by SDS-polyacrylamide gel electrophoresis, and blotted onto nitrocellulose. The blots were

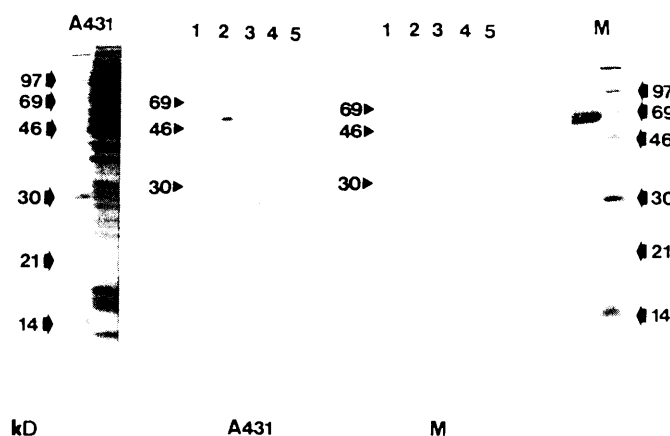


Figure 4. IgE antibodies from AD patients bind specifically to human proteins. Protein extracts from the epithelial cell line A431 and from tissue culture medium containing FBS (M) were separated by preparative SDS-polyacrylamide gel electrophoresis. Left and right, sections of the protein gels, which were stained with Coomassie Blue. The other portions of the gels were blotted onto nitrocellulose. In the center of the figure, sera from AD patients (lanes 1, 2), a myeloma supernatant containing greater than 2000 U/ml IgE (lane 3), serum from a nonatopic individual (lane 4), and buffer (lane 5) were probed with the nitrocellulose-blotted A431 extracts (A431) and medium components (M). Bound IgE was detected with ^{125} I-labeled anti-human IgE antibodies and visualized by autoradiography.

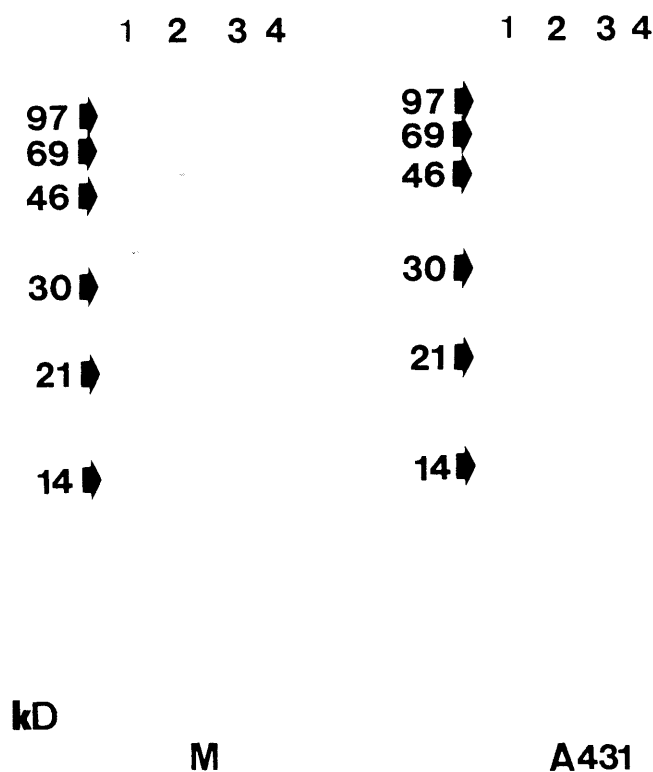


Figure 5. Preincubation of sera from AD patients with human proteins inhibits IgE binding to nitrocellulose-blotted human proteins. Sera from AD patients (lanes 1, 2), a nonatopic individual (lane 3), and buffer (lane 4), which were preabsorbed with nitrocellulose-blotted tissue culture components (M) or with nitrocellulose-blotted A431 proteins, were probed with blotted A431 extracts. Bound IgE was detected with ^{125} I-labeled anti-human IgE antibodies and visualized by autoradiography.

incubated with sera from AD patients (lane 1: total IgE >2000 kU/l; lane 2: >2000 kU/l; lane 3: 265 kU/l; lane 4: 1385 kU/l), a serum sample from a healthy nonallergic individual (lane 5: total IgE 27.7 kU/l), or with sera from RC patients without dermatitis (lane 6: total IgE 292 kU/l; lane 7: 503 kU/l). Sera from three of four AD patients (lanes 1, 3, 4) reacted with the PBMC extracts. These positive sera varied in their reaction patterns; each individual serum, however, identified equal-sized proteins in all three extracts. The sera from the RC patients without AD (lanes 6, 7), from

one AD patient (lane 2), and from the nonallergic healthy individual (lane 5) showed no significant IgE binding to PBMC proteins.

DISCUSSION

In 1916, Cooke and Van der Veer described hypersensitivity in 621 patients suffering from asthma after exposure to animals, pollen-induced seasonal hay fever, or urticaria and angioedema occurring after the intake of certain types of food. With the measurement of specific IgE antibodies, it became possible to attribute allergic symptoms to reactions of these antibodies with certain environmental antigens (allergens) present in pollens, mites, fungi, animal dander, or food (Johansson *et al.*, 1971). In certain patients with atopy, however, a causative link between allergic sensitization against environmental allergens and symptoms cannot be established, not even in the presence of hyperimmunoglobulinemia E.

The possibility that human proteins could serve as allergens gained support with the observation that AD patients frequently display immediate-type skin responses when tested with human dander extracts (Simon, 1947; Parish, 1960; Berrens, 1970). Human dander allergens were later described (Berrens, 1970), and the anaphylactic nature of allergy to human dander was confirmed by Prausnitz Küstner tests (Hampton and Cooke, 1941; Simon, 1944).

In the current study, we demonstrate that certain patients with atopy, particularly those with skin manifestations, have serum IgE antibodies against a variety of human proteins. No individual in the other study groups investigated (chronic idiopathic urticaria, SLE, GVHD, and healthy controls) had immunoblot-detectable IgE antibodies against human proteins, even though some of them had elevated serum IgE. It thus appears that the occurrence of IgE auto- or alloreactivity is linked primarily to the state of atopy rather than to the propensity of an individual to mount an IgE antibody response to a particular antigenic stimulus.

Although we detected IgE-defined autoantigens of similar molecular weight in different cells, certain extracts exhibited a restricted or even a differential expression pattern of IgE-binding moieties. The experimental design chosen in this study does not allow us to define whether the differences in the patterns of IgE auto- or alloreactivity among various cell extracts are due to the expression of different allo- or autoantigens in histogenetically different cell types or, alternatively, to differential degradation of proteins during the extraction procedure. The isolation of cDNAs coding for IgE-defined autoantigens from human expression libraries, as was already done for certain allergen-encoding cDNAs (Breitenbach *et al.*, 1990), followed by differential hybridization of these cDNAs with RNA from various cell sources will be necessary to clarify this issue.

It is possible that two different mechanisms are operative in the induction and maintenance of IgE auto- or alloreactivity. On one hand, the possibility exists that the IgE immune response is initiated by environmental allergens and is maintained by structurally related endogenous antigens. Alternatively, one could hypothesize that tissue injury results in the exposure and release of endogenous proteins which, after gaining access to professional antigen-presenting cells, could be presented to lymphocytes in an immunologically relevant fashion. In this context, it has been proposed recently that any antigen, i.e., altered self or self, can be presented to the immune system as long as the perturbation of tissue homeostasis leads to the activation and mobilization of professional antigen-presenting cells, e.g., dendritic cells (Matzinger, 1994). Such cells are abundant in the skin (Wang *et al.*, 1992; Lenz *et al.*, 1993) and respiratory tract (Holt *et al.*, 1990). This concept is in keeping with our observation that IgE allo- or autoreactivity occurs predominantly in AD, a highly pruritic skin disease prone to mechanical trauma, i.e., scratching.

One could speculate that auto- or alloreactive IgE antibodies play an important role in the pathogenesis of atopy, particularly AD. We postulate that, upon allergen-induced cross-linking, the binding of these antibodies to Fcε receptor-bearing cells results in biologically relevant signaling events. In the case of mast cells and basophils, the consequences are the production and release of the effector mole-



Figure 6. Donor-independent expression of IgE-defined autoantigens in PBMC extracts. Sera from AD patients (lanes 1–4), from one nonallergic individual (lane 5), and from two atopic individuals without dermatitis (lanes 6, 7) were probed for IgE reactivity with nitrocellulose-blotted proteins extracted from PBMC.

cules of allergic tissue inflammation (histamine, cytokines, eicosanoids, etc.). In the case of monocytes (Maurer *et al.*, 1995), dendritic cells (Mudde *et al.*, 1990; Maurer *et al.*, 1996), and B cells (Kehry and Yamashita, 1989), IgE-facilitated binding and presentation of autoantigens will ultimately lead to the perpetuation and amplification of the "auto"-allergic response. Should further experiments provide additional evidence for this concept, interference with production and target binding of autoreactive IgE antibodies could be a promising strategy for the treatment of AD patients.

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