

Absence of specific IgE antibodies in allergic contact sensitivity to formaldehyde

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Immunologic reactions are customarily divided into two broad categories, cell-mediated and antibody-mediated. An interplay between these two pathogenetic principles is indicated by reactions such as cutaneous basophil hypersensitivity, late-phase reaction, and cutaneous lesions indistinguishable from regular allergic contact dermatitis lesions after sensitization with IgE antibodies against certain haptens. In the present study, 23 patients with a history of a positive epicutaneous test to formaldehyde participated. On retest, 15 showed a positive reaction. Eight patients were Phadiatop[®] positive, indicating an atopic diathesis, and eight had a history of or ongoing atopic dermatitis. On RAST test[®], only two, nonatopic patients had specific IgE antibodies to formaldehyde. In the cellular infiltrates of biopsies from epicutaneous test sites, cells reactive with monoclonal antibodies against IgE were found in positive and negative formalin tests, both in atopics and nonatopics, as well as in control biopsies from nonlesional skin.

Double immunofluorescence staining experiments showed that IgE occurred on Langerhans' cells. The proportion of IgE-positive cells correlated to the level of serum IgE, but not to atopy. These cells were also found both in the epidermis and in the dermis in nonatopic patients. ICAM-1 occurred on keratinocytes in all patient groups. This study does not support the hypothesis that specific IgE antibodies are active in the pathogenesis of contact sensitivity to formaldehyde either in atopic or in nonatopic patients.

Experimental contact hypersensitive reactions to dinitrofluorobenzene (DNFB) are mediated by monoclonal IgE antidinitrophenyl (DNP) antibodies in mice (14); the results of the ear swelling test used were indistinguishable from the test reaction obtained in mice sensitized by conventional epicutaneous application of antigen. Other delayed reactions with similarities to allergic contact dermatitis (ACD) and mediated by immunoglobulins have also been described. One example is cutaneous basophil hypersensitivity, which is characterized by a prominent infiltrate of basophils (1, 15). It has been transferred passively in guinea pigs with IgG1 or IgE antibodies (2). Another example is the late-phase reaction, which in the skin is an IgE-dependent reaction characterized by erythema, edema, and a mixed inflammatory cell infiltrate with a significant component of mononuclear cells, after an initial wheal and flare reaction (8, 16).

All these data raise the question of whether antibodies are also important in ACD in man. The biphasic pattern with both an immediate and a late reaction (14) might be particularly relevant to atopic dermatitis. In this condition an immediate irritation can be seen upon contact with certain allergenic substances, and this irritation is followed by a delayed but more persistent eczematous lesion.

The aim of the present study was to look for specific IgE antibodies in the serum of patients with epicutaneous reactions to formaldehyde, and also to characterize the cellular infiltrate of these reactions.

Patients and methods

Patients

Twenty-three patients with positive epicutaneous test reactions to formaldehyde were recruited from

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three dermatologic departments in Sweden. The patients were 21–74 years old (mean 39); 19 were women. The tests had been performed 6 months to 10 years before inclusion in the study.

The study was approved by the Ethics Committee of the Karolinska Hospital, Stockholm.

Patch testing

The patients were interviewed regarding personal history of atopic disease (eczema, asthma, or rhinitis). A blood sample was collected and serum was kept frozen at -20°C pending analysis. The patients were classified as atopic if they had a history of atopic disease, a serum IgE level higher than 120 kU/l (geometric mean $+2$ SD of a normal population), or a positive atopy screening test result (Phadiatop[®], Pharmacia Diagnostics AB, Uppsala, Sweden).

The test material was 1% formaldehyde in water (Chemotechnique Diagnostics AB, Malmö, Sweden) applied in Finn Chambers[®] (Epi-test OY, Helsinki, Finland). The tests were applied on the back for 48 h and read at 72 h. In three patients, additional tests were also applied for 3 and 27 h before reading. Evaluation criteria were designated as follows:

- negative test
- ? questionable reaction; faint erythema only
- + weak positive reaction; erythema, infiltration, discrete papules
- ++ strong positive reaction; erythema, infiltration, papules, vesicles
- +++ extreme positive reaction; intense erythema, induration, coalescing vesicles.

Skin biopsies

Punch biopsies 3 mm in diameter were taken from the test sites after local anesthesia of the perilesional skin with lidocaine 20 mg per ml (without epinephrine). Control biopsies were taken from sites exposed to empty Finn Chambers applied for 48 h and also from an untreated site. All biopsies were taken from nonlesional skin which was clinically healthy-looking before the test. The biopsies were kept in Histocon[®] (Histolab, Gothenburg, Sweden) at $+4^{\circ}\text{C}$ until they were snap-frozen in chilled isopentane and stored at -70°C .

Peroxidase-antiperoxidase staining. Acetone-fixed, 6- μm -thick, cryostat sections of the biopsy specimens were stained by a three-step monoclonal antibody peroxidase-antiperoxidase (PAP) method (17). Endogenous peroxidase was blocked by incubation in 0.3% H_2O_2 in phosphate-buffered saline (PBS) for 15 min, and then the sections were allowed to react with normal rabbit serum (diluted 1/10) for 10 min to reduce nonspecific staining. Next, the sec-

tions were incubated with the following mouse monoclonal antibodies: 1) a combination of two antibodies against human IgE of two different specificities (anti-IgE-D1 and -D2 antibodies; working dilution 1/160; donated by Dr CGM Magnusson, Department of Clinical Immunology, Karolinska Hospital, Stockholm, Sweden), 2) an antibody against ICAM-1 (anti-ICAM-1/CD54 antibodies (84H10); working dilution 1/80; Serotec, Oxford, UK); 3) Leu-4 (CD3, pan T cells; working dilution 1/256; Becton Dickinson, Sunnyvale, CA, USA); and 4) anti-HLA-DR (activated T cells, B cells, macrophages, monocytes, Langerhans' cells; working dilution 1/128; Becton Dickinson). Rabbit antimouse immunoglobulin (diluted 1/40; Dakopatts, Copenhagen, Denmark) was used as a secondary antibody. Finally, the sections were allowed to react with preformed complexes of horseradish peroxidase and monoclonal antihorseradish-peroxidase antibodies (diluted 1/250; Dakopatts). The peroxidase reaction was developed with 3-amino-9-ethylcarbazole, and the sections were counterstained with Mayer's hematoxylin. Specificity tests included omission of the primary antibodies, and staining was not observed in these tests. At least two sections per antibody and biopsy were examined. Estimation of the total amount of reactive cells per section was based on a semiquantitative scale ($-$ = no, $+$ = few, $++$ = moderate, and $+++$ = many positive cells). Reactive cells in the epidermis were estimated separately from positive cells in the dermis, but for the anti-ICAM-1 antibody, only epidermal staining was assessed. The scale was adjusted so that the scale step "many" ($+++$) referred to the maximal number of cells reactive to each antibody within all the specimens.

Each biopsy specimen was also stained with hematoxylin and eosin for estimation of the inflammatory response, which was graded as small ($+$), moderate ($++$), or large ($+++$) for dermal cell infiltrates and the presence of epidermal vesicles.

Double immunofluorescence staining. Double immunofluorescence staining was performed on those biopsy specimens taken at 72 h in which IgE + epidermal cells were found in immunoperoxidase staining (Table 1). Frozen, acetone-fixed, 6- μm -thick sections were incubated with the combination of mouse monoclonal anti-IgE-D1 and -D2 antibodies (see above; working dilution 1/10) for 30 min at room temperature, followed by washes in PBS. The sections were then allowed to react with fluorescein-isothiocyanate (FITC)-labeled rabbit antimouse immunoglobulin (working dilution 1/20; Dakopatts) for another 30 min. Normal mouse serum was used as a blocking agent, and then the sections were incubated with phycoerythrin (PE)-labeled anti-CD1a antibodies (T6/RD1, staining Langerhans' cells;

Table 1. Results from analyses of 23 patients with a previous positive patch-test reaction to formaldehyde. CAP form. indicates specific antibodies against formaldehyde given as RAST class/kU_A/l

Pat. no.	CAP form.	Phadiatop [®]	Atopy*	sIgE kU/l	Patch-test reaction at 72 h	IgE+ cells in epid./dermis**				N
						72-h F	72-h C	27-h F	3-h F	
1	1/0.4	-	0	230	+++	+++/>+++	-/>++	+/>++	-/>++	+/>++
2	2/0.9	-	0	28	+++	+/>++	-/>++	-/>+	-/>+	-/>+
3	-	+	AD	558	++	+++/>+++	+/>++			
4	-	+	0	366	++	+++/>+++	+++/>+++			
5	-	+	0	100	+++	+/>++	-/>+			-/>+
6	-	+	AD	2135	-	+++/>+	+++/>+			+++/>H
7	-	+	AD	608	-	+++/>+	+++/>+			
8	-	+	AR	178	-	-/>+	+/>+			+/>+
9	-	+	AD	63	-	+/>+	-/>+			-/>+
10	-	+	AD	15	-	-/>-	-/>-			-/>-
11	-	-	AA	264	++	+++/>+++	+/>++			
12	-	-	AD	33.2	+	+++/>+	-/>+			
13	-	-	0	31	++	-/>++	-/>+	-/>-	-/>+	-/>-
14	-	-	AD	11.3	+	-/>+	-/>+			
15	-	-	0	10.7	+++	+++/>+	-/>+			
16	-	-	0	10.3	++	-/>++	-/>-			
17	-	-	0	6.1	++	-/>+	-/>-			
18	-	-	0	4.8	++	-/>-	-/>-			
19	-	-	0	3.6	++	+/>+	-/>+			
20	-	-	0	2.8	++	-/>+	-/>-			
21	-	-	0	64	-	-/>+	-/>+			-/>+
22	-	-	AD	22.8	-	+/>+	-/>+			
23	-	-	0	21	-	-/>-	-/>-			-/>+

* AD=atopic dermatitis; AR=allergic rhinitis; AA=allergic asthma; F=formaldehyde patch-test biopsy; C=control skin biopsy, empty chamber; N=control skin biopsy, nonoccluded.

** - =no IgE+ cells (epidermis/dermis); + =few IgE+ cells (1-5/1-20); ++ =moderate IgE+ cells (6-20/21-200); +++ =many IgE+ cells (>20/>200); H=hair follicle.

working dilution 1/20; Coulter Immunology, Hi-aleah, FL, USA). The sections were mounted with glycerol in PBS containing *p*-phenylenediamine to reduce stain fading during microscopy (10). This double-staining method permitted the identification of IgE and CD1a in the same section when the slides were analyzed in a Leitz Orthoplan fluorescence microscope with epiillumination and interchangeable filter combinations for the fluorochromes. The sections were compared with those stained only for CD1a or IgE.

Serum IgE and IgE antibodies. The serum IgE and IgE antibody concentration expressed in kU/l and kU_A/l was determined by Pharmacia CAP System IgE and RAST, respectively, and the atopy diagnosis was evaluated by Pharmacia CAP System Phadiatop and given as positive or negative. All three tests were performed according to the manufacturer's recommendations (Pharmacia Diagnostics AB, Uppsala, Sweden). On the basis of case history, relevant allergens were selected from the available panel. In addition, each serum sample was tested for IgE antibodies to formaldehyde (k80). No background, nonspecific IgE-binding (<0.35 kU_A/l) was found in CAP for any allergen at serum IgE concentrations below 3000 kU/l.

Results

Serologic data

Ten patients (43%) were classified as atopic on the basis of a high serum IgE level or a positive Phadiatop. Only two patients had a positive CAP test to formaldehyde; in both cases, the IgE antibody concentrations were low (Table 1). Neither patient was atopic.

Patch test reactions and skin biopsy specimens. Of the 23 patients with previous positive patch test reactions to formaldehyde, 15 showed positive reactions at 72 h at the retest in the present study (Table 1). On histologic examination all those with a positive patch test reaction and two additional patients with a negative test reaction had small-to-large dermal mononuclear cell infiltrates as graded by hematoxylin and eosin staining. Nine of these biopsy specimens displayed epidermal vesicles. Four of the 23 biopsies obtained from empty patch test reactions had small dermal cell infiltrates. The rest appeared to be normal, as did the 10 biopsies taken from nonoccluded control skin. In the three patients in whom biopsy specimens were taken from formaldehyde patch test areas at 3 h and 27 h, two patch test reactions were positive at 27 h, showing small dermal cell infiltrates.

Most of the infiltrating cells, mainly localized

perivascularly, expressed CD3 and HLA-DR. Only occasionally were CD3+ T cells observed in the epidermis in control skin, with a slight increase in 7/23 specimens from skin occluded with empty Finn Chambers. Moderate-to-large dermal CD3+ cell infiltrates in positive patch test reactions were usually accompanied by an equal increase of CD3+ cells in the epidermis. HLA-DR expression on keratinocytes was observed only in two specimens from positive patch test reactions taken at 72 h. In both cases, staining was limited to the basal cell layers of hair follicles.

In contrast, ICAM-1 expression on keratinocytes was observed in most specimens (13/15) taken from positive formaldehyde reactions at 72 h and in the three taken at 27 h. Biopsies with many ICAM-1+ keratinocytes were usually associated with large dermal cell infiltrates. ICAM-1+ keratinocytes were also present in 6/8 biopsies from negative formaldehyde reactions, in 10/21 specimens from skin occluded with empty Finn Chambers, and in 5/10 control skin specimens. In these cases, positive staining was usually limited to a few basal keratinocytes or small groups of keratinocytes.

The two patients with IgE antibodies against formaldehyde showed IgE+ dendritic epidermal cells and, in epidermal vesicles, also round IgE+ cells in the specimens taken at 72 h from the positive patch-test reactions (Table 1). In patient no. 1, IgE+ epidermal cells were also seen in the biopsy taken at 27 h and in normal-looking skin (Table 1). Dermal dendritic or round IgE+ cells were found scattered in all cases; most frequently, in the positive patch test reactions at 72 h. IgE+ cells within both the epidermis and the dermis were found in 14 patients. Six of them had a history of, or ongoing, atopic dermatitis, one had atopic asthma, and one had atopic rhinitis. Thus, 6 of the 14 patients were not atopic. Two patients with atopic dermatitis did not show the combined pattern of IgE+ cells in both epidermis and dermis. Eight of the 23 patients had a history of, or ongoing, atopic dermatitis. Their IgE levels covered a wide range from 11.3 to 2135 kU/l. Five of them were Phadiatop positive and three were patch-test positive to formaldehyde.

Of the eight Phadiatop-positive patients, three had positive patch-test reactions to formaldehyde. Biopsy specimens from these patients had a similar appearance, as regards IgE+ cells, to those from the patients with IgE antibodies to formaldehyde. The five patients with negative patch-test reactions displayed IgE+ epidermal and dermal cells, with the highest frequency in the two with the highest IgE levels (Table 1). Only the patient with the lowest IgE level (15 kU/l) had no detectable IgE+ cells in the skin.

Of the 13 patients who were neither Phadiatop

positive nor CAP positive for formaldehyde, 10 had positive patch-test reactions to formaldehyde. IgE+ epidermal cells were observed in five of these patients with sIgE levels of 3.6–264 kU/l, but not in those five patients with sIgE 2.8–31 kU/l (Table 1). In no case were IgE+ cells found within the epidermis alone, but always associated with their presence in the dermis (Table 1). IgE+ epidermal cells in normal, nonoccluded skin were found only in 3 of 10 patients, with IgE values of 178, 230, and 2135 kU/l, respectively. Six of 23 biopsies from occluded control skin had IgE+ cells in the epidermis, all from patients with IgE levels above 178 kU/l (Table 1). Double immunofluorescence staining revealed that when IgE+ epidermal cells were observed with immunoperoxidase staining in the specimens, CD1a+ and IgE+ epidermal cells were also observed. The double-positive epidermal cells were more frequent and easier to detect in biopsy specimens from patients with raised IgE levels and a positive patch-test reaction.

Discussion

IgE antibodies in serum specific to formaldehyde were found in only two of the 15 patients with positive patch tests. These two had no clinical signs of atopy, and their contact sensitivity was relevant and strong, as shown by the patch tests. The presence in serum of detectable levels of IgE antibody is, in atopic allergic diseases of the airways, a reliable sign of a clinically relevant sensitization (9). Formaldehyde is not a typical allergen; most of the airway reactions are due to its highly irritable nature. However, rare cases of IgE-mediated allergy to formaldehyde have been described in occupational settings, and such allergy is not uncommon after blood-borne exposition at hemodialysis with formaldehyde-sterilized equipment (12). Of the eight patients with atopic dermatitis, only three were positive on the repeat patch testing, and none of them had specific IgE antibodies; this was also true of the eight Phadiatop-positive patients.

A very attractive hypothesis of the pathogenesis of atopic dermatitis entails the effect of specific IgE antibodies on effector cells in skin. The occurrence of cells with surface IgE in both epidermis and dermis and in both atopic and nonatopic skin confirms previous findings (5). No differences in number or distribution of such cells were seen among patients with or without atopic dermatitis or between Phadiatop-positive and Phadiatop-negative patients. This contrasts with the recent report of Ward et al. (20) showing that IgE+ cells when found within both epidermis and dermis were indicative of atopy. We also detected IgE+ cells in patients with very low serum IgE levels, although in low numbers.

These data suggest that the anti-IgE antibodies used in the immunohistology of this study have a high affinity for IgE. The serum IgE level seemed to influence the number of cells carrying IgE, and this agrees with previous reports (4, 5, 18). The results of the double staining, with antibodies defining Langerhans' cells and IgE, indicate that at least part of the IgE+ cells are Langerhans' cells. This also agrees with two recent reports showing the presence of high-affinity receptors for IgE on normal human epidermal Langerhans' cells (6, 19).

These results do not exclude a pathogenetic role for IgE in ACD. The monoclonal antibodies against IgE used in the present experiment are not specific for a putative IgE antibody against formaldehyde. The IgE-positive skin cells may be covered with a range of IgE antibodies against various antigens, of which formaldehyde represents a minority not detected by our methods. The proportion of patients positive for formaldehyde on the secondary, repeat test was 65%. This figure may seem surprisingly low. However, current work shows that patients with positive patch tests to formaldehyde tend to lose their reactivity with time (unpublished), a result which is consistent with loss of sensitivity to lanolin, for example (7). The reliability of the patch-test method is not optimal, but this does not seem to be the sole explanation of the lack of concordance between the two test occasions.

The occurrence of CD3-positive cells in the biopsies tallies with the well-known preponderance of T cells in most inflammatory infiltrates in skin. ICAM-1 (intercellular adhesion molecule-1)-positive keratinocytes can be found in inflammatory dermatoses characterized by an accumulation of T lymphocytes (3, 13) and can also be seen in normal skin (11). This expression on keratinocytes is stimulated by interferon-gamma and tumor necrosis factor-alpha and -beta (13). In our present study, ICAM-1+ keratinocytes were found in most patch-test reactions, reflecting increased inflammatory activity in the skin.

The present data thus give no support to the hypothesis that specific IgE antibodies contribute to ACD from formaldehyde, even in atopic patients. Even if it cannot be excluded that low, undetectable levels of such antibodies in serum or locally in tissue might have been present in our patients, our final conclusion must be negative.

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