

Prevalence and Functional Role of Anti-IgE Autoantibodies in Urticarial Syndromes

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The prevalence of autoantibodies of immunoglobulin G (IgG) and immunoglobulin M (IgM) classes directed against myeloma immunoglobulin E (IgE) were determined in distinct subsets of urticaria, using an enzyme immunoassay. IgG anti-IgE antibodies were found in five of nine patients (55%) with cold urticaria, four of eight patients (50%) with urticarial vasculitis, and three of six patients (50%) with chronic urticaria. IgM anti-IgE antibodies were found exclusively in cold urticaria (two of nine patients, 22%). Heating of these

sera increased the binding to IgE, suggesting immune complex formation. Several positive sera were capable of inducing histamine release from normal peripheral basophils and caused a wheal-flare response upon intradermal injection. Sera containing such autoantibodies from three cold urticaria patients were studied for passive transfer of cold sensitivity. One serum containing IgG anti-IgE gave a strongly positive transfer test at 5 h but not 48 h, suggesting a pathogenic role for the IgG. *J Invest Dermatol* 90:213-217, 1988

Autoantibodies directed against IgE have previously been described in several conditions, mostly related to atopy [1-6]. The interest in such autoantibodies stems from their potential to initiate histamine release from dermal mast cells or basophils and to participate in the pathogenesis of these disorders. We therefore developed an enzyme immunoassay to ascertain the prevalence of anti-IgE antibodies of both IgG and IgM classes in urticaria and studied the ability of sera containing these autoantibodies to cause histamine release. Our data indicate a high incidence of these antibodies in cold urticaria and in urticarial vasculitis and we consider the possible role of anti-IgE immunoglobulins in the development of urticarial lesions.

METHODS

Sera and Patients Sera were randomly collected from patients seen by the allergy-immunology service with urticarial disorders. Patients with chronic idiopathic urticaria as defined previously [7], cold urticaria as documented by history and ice-cube challenge [8], and urticarial vasculitis with hypocomplementemia and consistent skin biopsies [9] comprised the study group. Healthy age-matched nonatopic individuals served as controls. Sera were stored at -20°C prior to the assay. Patients with cold and chronic urticaria were

negative for rheumatoid factor by latex fixation. Total IgE levels were determined by radioimmunosorbent (PRIST) assay (Pharmacia Lab, Piscataway, New Jersey). Total serum IgM levels were determined by radial immunodiffusion (Calbiochem Diagnostics, San Diego, California).

Enzyme Immunoassay This assay to determine anti-IgE antibodies was performed with minor modifications as described by Nawata, et al [2]. Myeloma IgE (provided kindly by Dr. K. Ishizaka) was bound to multiwell polystyrene plates (Dynatech Inc., Alexandria, Virginia) at a final concentration of 1 µg/well in bicarbonate coating buffer (pH 9.6). Reactive sites were then blocked with 1% bovine serum albumin and the plates stored at 4°C. Serum was then added in PBS-tween at 1:10 or 1:100 dilution and the mixture incubated overnight. After extensive washing, 100-µl alkaline phosphatase labeled anti-human IgG or IgM (Fc specific, Cappel Laboratories, Malverne, Pennsylvania) at 1:1000 dilution was added, incubated for 2 h, and then reacted with 100 µl of the substrate, *p*-nitrophenyl phosphate (1 mg/ml). The optical density (OD) at 405 nm was determined at 30 and 60 min on a Dynatech ELISA reader. The quantity of reactive IgG or IgM was calculated using standard amounts of purified IgG (Miles Laboratories) or IgM (Calbiochem, Summerville, New Jersey) ranging from 10 to 1000 ng bound directly to the plates. The data were then expressed as a ratio of OD 405 nm (sample)/OD 405 nm (standard) × (ng of standard).

Specificity Studies A series of immunoadsorption experiments were performed to determine the specificity of the enzyme immunoassay. Sepharose 4B (Pharmacia, Piscataway, New Jersey) activated with cyanogen bromide [10] was coupled to myeloma IgE, rabbit anti-human IgG, rabbit anti-human IgM (Calbiochem) or Staph protein A (Pharmacia). Reactive sera were exposed to each of these sepharose preparations (1:1 v/v) and rocked overnight at 4°C in microfuge tubes. The sepharose was then sedimented at 200 G for 10 min and the sera were retested on the EIA. For certain experiments, the adsorbed material was washed and then eluted with 0.5M acetic acid and neutralized. The eluates were then tested on

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Abbreviations:

EIA: enzyme immunoassay
IgE: immunoglobulin E
IgG: immunoglobulin G
IgM: immunoglobulin M
OD: optical density

the EIA. In addition, competitive inhibition experiments were performed by preincubating reactive sera with increasing concentrations of IgG, IgA, IgM, and IgE and then tested on the EIA. Finally, sera were heated to 56°C for 30 min in an attempt to alter the antigen determinants in the IgE Fc region [11] and thereby dissociate the complex.

Basophil Studies Whole blood was obtained from normal non-atopic volunteers in 10% 0.2M EDTA. Leukocytes were separated by dextran sedimentation (0.3% dextran, 0.3% glucose, 0.15M NaCl) at 37°C, in a standard manner [12]. Rabbit anti-IgE was prepared as previously described [13]. Human serum reactive on the EIA (as above) or gamma globulin eluted from Staph protein A-sepharose 4B was used to stimulate the basophils for 30 min, at 37°C in HEPES buffer containing 0.03% human serum albumin, 1.5 mM CaCl₂, and 2.0 mM MgCl₂. Histamine was assayed using the radioenzyme assay of Beaven et al [14].

Skin Test Studies In order to study the effects of these antibodies on cutaneous mast cells, serial dilutions of reactive sera were used for prick testing of normal volunteers and the wheal-flare response recorded. Undiluted sera from three cold urticaria patients were tested for their ability to passively sensitize normal volunteers [15]. Ice cube challenges were then performed at separate sites after 5 and 48 h. Passive transfer of serum from a normal volunteer was utilized as a negative control with each assay performed. Responses were quantitated by measuring the area of erythema and wheal at 10 min, and 24 and 48 h; the responses were graded 0 to 4 (+) as described by Norman [16]. Control tests included both normal sera and diluent alone.

Statistics The product moment correlation coefficient (*r*) was obtained by the method of Pearson [17].

RESULTS

Enzyme Immunoassay The prevalence of IgM and IgG autoantibodies directed towards IgE are shown in Figs 1A and 1B. Each point represents the mean of duplicates with less than 5% variance. Each sample was reassayed on at least three separate occasions and less than 10% interassay variability was observed. Positivity represents binding greater than 2 standard deviations (SD) above the mean of the control group. Of the cold urticaria patients studied,

55% had IgG anti-IgE antibodies, whereas only 2/9 (22%) had IgM reacting with IgE. Only one of these sera had both classes of autoantibodies. Associated atopic disease was absent by history in the positive group with one exception (bronchial asthma). The remainder of the positive sera in urticarial vasculitis (50%) and chronic urticaria (50%) were found to be solely of the IgG class. In general, the positive chronic urticaria sera were reactive just above the 2 SD confidence level, but the mean for the entire group did not significantly differ from controls ($p > 0.05$). The total serum IgE levels were within the normal range (20–600 IU) for the entire group, with one exception (711 IU). There was no correlation between total IgE levels and anti-IgE antibodies in any group studied ($p > 0.1$). Serum IgM levels were within the normal range in all patients, including the two with IgM anti-IgE activity.

The specificity of the EIA was confirmed by immunoadsorption of several strongly positive sera (see Methods). The results of representative experiments using sera positive for either IgG or IgM anti-IgE are shown in Fig 2. The IgM anti-IgE reactivity was significantly reduced by passage over IgE-sepharose and was essentially abolished by the anti-IgM immunoadsorbent. The IgG anti-IgE reactivity was markedly reduced by interaction with IgE sepharose, Staph protein A-sepharose, or the anti-IgG immunoadsorbent. The acetic acid eluate of the Staph protein A column recovered the anti-IgE reactivity. Further confirmation of specificity included the absence of inhibition if positive sera for IgM anti-IgE were passed over anti-IgG sepharose; IgG anti-IgE sera were passed over anti-IgM sepharose; or either was passed over pooled IgG sepharose (data not shown). In addition, the competitive inhibition experiments confirmed the specificity of the sera with IgM and IgG antibodies against IgE because no reduction in EIA reactivity was found after preincubation with IgM, IgG, or IgA with final concentrations as high as 0.1 mg/ml. However, IgE inhibited greater than 50% of the activity at concentrations at or above 10 µg/ml. The results of the EIA after heating the sera at 56°C for 30 min to denature IgE are shown in Table I. In general the reactivity of the cold urticaria and urticarial vasculitis sera increased when compared with the sera taken from the controls. No specific pattern emerged with respect to the IgG or IgM class of anti-IgE activity. The mean percent increase was 112% for the patients with cold urticaria and 66% for the patients with urticaria vasculitis. The normal sera, however, decreased by a mean of 17.4%.

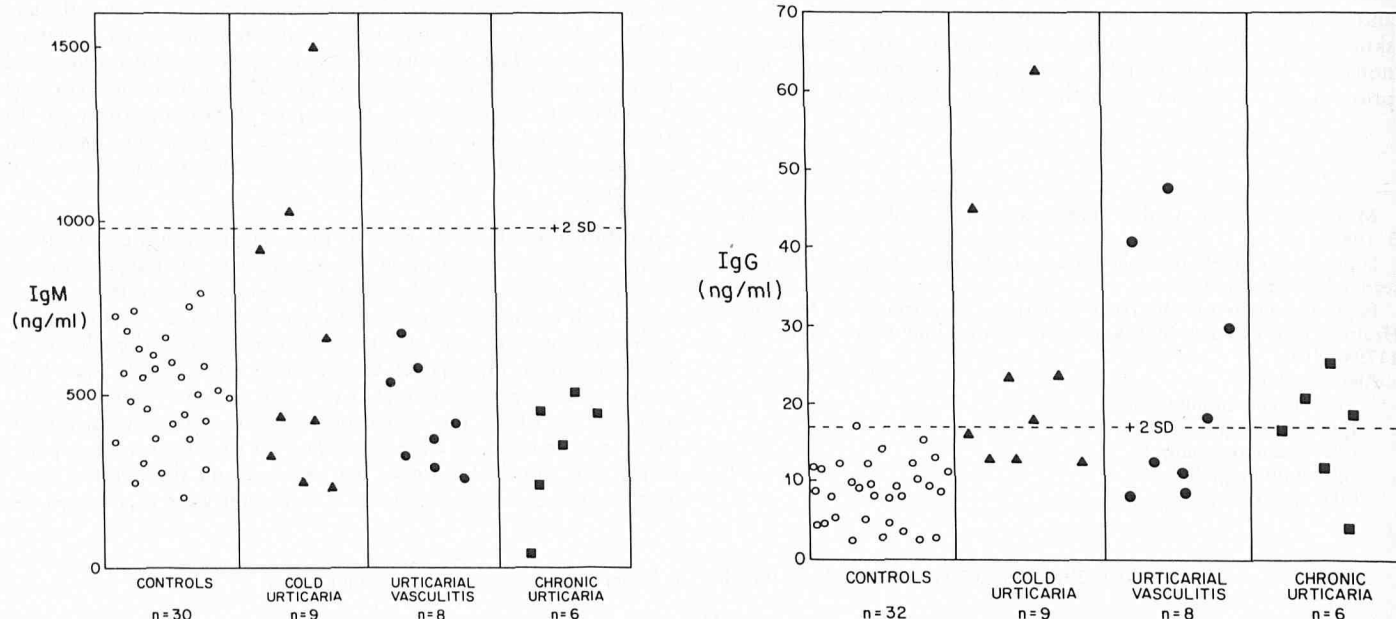
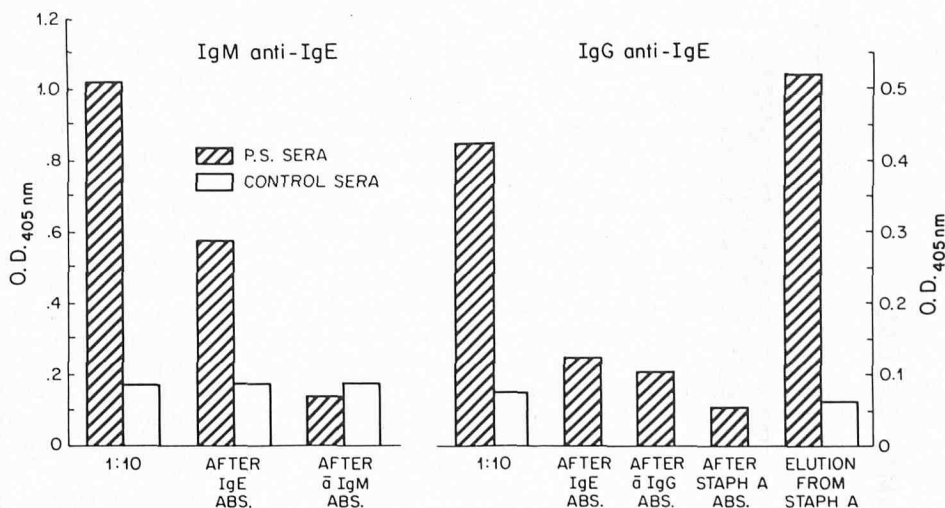


Figure 1. (A: left chart) IgM antibodies as measured by the enzyme immunoassay. (B: right chart) IgG anti-IgE antibodies as measured by the enzyme immunoassay. Positive samples are defined as > 2 SDs from the control values (dotted line).

Figure 2. The left half represents the optical density (OD 405 nm) of a reactive (IgM anti-IgE) serum and a control serum before and after incubation with IgE myeloma coupled to Sepharose as well as anti-IgM coupled to sepharose. The right half represents the optical density of a reactive (IgG anti-IgE) serum and a control serum before and after incubation with an IgE-sepharose absorbant, anti-IgG-sepharose and Staph protein A-sepharose as well as the eluate from the Staph protein A-column.



The results obtained from the tests utilizing polystyrene plates that were not initially blocked with bovine serum albumin were not significantly different from above. The presence of antibodies directed to bovine albumin were not detected in these patients.

Basophil Studies The net histamine release (HR) from normal basophils exposed to serial dilutions of a cold urticaria serum with high titer IgM anti-IgE antibodies is shown in Fig 3. The total histamine was 60 ng/ml and spontaneous release was less than 2%. Evidence that the releasing activity may be related to IgM binding to IgE is shown by significant diminution of HR following passage over IgE sepharose or an anti-IgM immunoabsorbent. No such decrease was seen if the serum was passed over IgG sepharose. The remainder of the five positive sera (by EIA) yielded low-grade HR (<12%), and this varied according to the specific donor of the basophils. In an attempt to simulate the ice cube challenge test, the basophils exposed to sera (diluted 1:100) containing anti-IgE antibodies were placed at 4°C for 15 min, rewarmed to 37°C, and the HR was determined. No consistent pattern emerged, although enhancement of HR was seen in 5 out of 11 experiments using 3 positive sera and no enhancement was seen in control sera.

Passive Sensitization Studies Attempts to passively sensitize normal individuals with high titer cold urticaria sera met with variable success. No immediate wheal and flare was obtained when these sera were initially injected. A cold challenge was then per-

formed after 5 and 48 h. As indicated in Table II, serum from patient 1 contained IgM anti-IgE autoantibodies as measured by the EIA and was found to transfer cold sensitivity to 1 of 3 individuals at 5 h, but not at 48 h. Patients 2 and 3 contained IgG anti-IgE autoantibodies in the serum. Both patients were positive (using 2 recipients) at 5 h and serum from patient 3 gave sustained cold reactivity at 48 h.

DISCUSSION

Anti-IgE autoantibodies have been demonstrated previously in a variety of pathologic conditions relating to atopy, arthritis or, at times, arising naturally [1-6]. The functional significance of these antibodies, however, has remained generally unexplored. We therefore undertook this study to investigate the frequency and cellular effects of these autoantibodies in patients manifesting distinct subsets of urticaria.

We have documented the presence of IgG antibodies directed towards myeloma IgE in approximately two-thirds of patients with cold urticaria, and in one-half of the patients with chronic urticaria or small vessel vasculitis presenting with urticaria. In addition, we found two patients who produced significant amounts of IgM anti-IgE antibodies in association with cold urticaria. Previously, Inganas et al [4], in an extensive study of anti-IgE antibodies, reported that two out of six patients with cold urticaria were found to have IgG anti-IgE antibodies.

Table I. Effect of Heating^a on Enzyme Immunoassay

Cold Urticaria		Urticarial Vasculitis		Controls	
Patient	% Change ^b	Patient	% Change	Patient	% Change
TR	+58	GT	+48	PN	+14
AB	+87	PO	+105	JB	-26
AD	+22	TB	+47	LA	-24
ES	+189	PI	+50	AK	-47
JT	-6	NF	+76	BG	-21
EP	+167	AC	+40	JL	-24
PL	+26.9	JP	+97	JS	+4
MC	-25.7			PG	-2
PS	+487.5			GH	+22
				JP	-70
Mean	+111.7		+66.1		-17.4%

^a Serum (1:10 dilution) was heated to 56°C for 30 min and OD 405 nm was compared to unheated samples.

$$^b \text{ \% Change} = \frac{\text{OD}_{30'} \text{ heated} - \text{OD}_{30'} \text{ norm}}{\text{OD}_{30'} \text{ norm}} \times 100$$

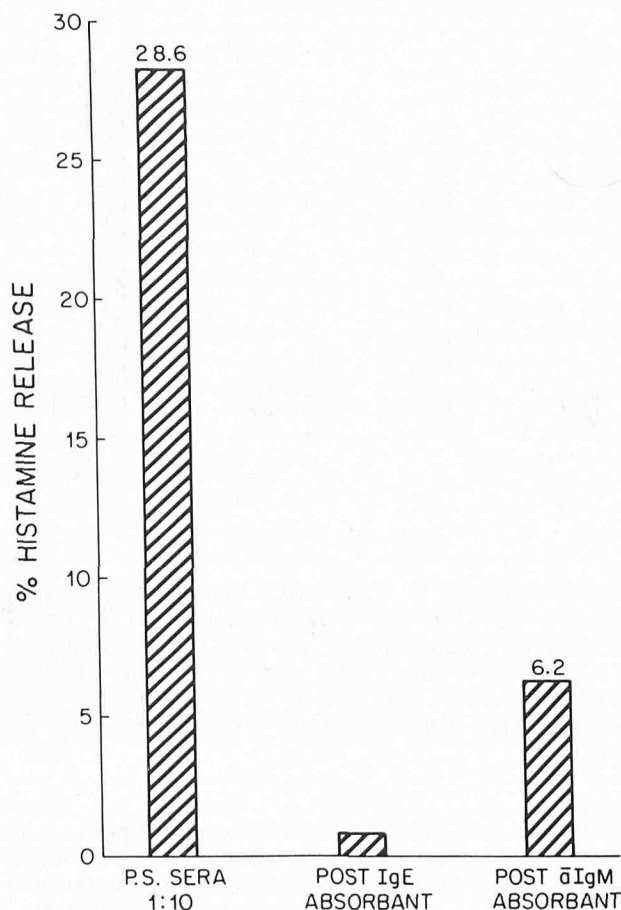


Figure 3. Histamine released from basophils following incubation with a cold urticaria serum P.S. containing high titer IgM anti-IgE antibodies. The effect of incubation with either IgE-sepharose or anti-IgM immunoabsorbant is shown.

The specificity of the enzyme immunoassay as modified from Nawata et al [1] was confirmed by a series of immunoabsorption experiments. Although it is conceivable that other IgE-binding factors that have been recently described [18] might bind to the solid-phase IgE and then to the conjugated indicator in this assay, it is unlikely that these factors have such heterologous affinity. In addition, the eluted material from Staph protein A-sepharose contained exclusively IgG as seen by SDS-polyacrylamide gel electrophoresis (data not shown). This material reacted in the enzyme immunoassay in the same manner as the original serum.

The heating of sera to cause an increase in reactivity on our assay (Table I) suggests that the antibodies may circulate as complexes and the antigenic determinant depends on the heat-labile intact CH₂ domain along the ε-Fc chain [4,5,11]. In separate experiments (manuscript in preparation) we have analyzed the reactivity of autoantibodies to IgE and found them to be reactive with the Fc

region. At this point, we therefore have no evidence that these antibodies react towards the Fab fragment or behave as anti-idiotypes.

In an attempt to explore the functional role of these autoantibodies, we utilized both peripheral leukocytes and skin testing to study histamine release from basophils and mast cells. We observed dose-dependent histamine release from normal basophils from certain individuals after exposure to reactive sera and no release with control or unreactive sera. No histamine release is obtained upon incubation of any patients sera with their own basophils as has been observed previously [8]. In some experiments, we found enhanced release after cooling and rewarming the basophils by using cold urticaria sera containing anti-IgE antibodies. However, no clear temperature dependent mechanism emerged. Furthermore, the majority of the serum induced low grade histamine release, similar to previous reports in other conditions [3,6]. The sera that were heated to dissociate complexes (and then demonstrated increased anti-IgE activity) did not significantly change with respect to its ability to release histamine. As anti-IgE immunoglobulins may circulate as immune complexes, it is possible that histamine release obtained upon incubation of sera and basophils is due to generation of anaphylatoxins as a result of complement activation. The present studies do not exclude this possibility, although the reactivity of heated sera suggests a non-complement-dependent reaction.

Several cold urticaria sera were capable of transferring cold sensitivity to normal individuals. In one, the transfer was positive at 5 h but not 48 h and this serum had a high titer IgG anti-IgE. It is possible that this IgG autoantibody is pathogenically significant. However, it is not clear why a discrepancy exists between the passive sensitization studies and the basophil release studies. Differences in mast cell and basophil responsiveness to certain secretagogues have been reported, but these usually do not involve the IgE system. The exception is cold urticaria where IgE dependent mast cell secretion is demonstrable while basophils fail to secrete under the same conditions [8]. Passive transfer of cold urticaria by IgM with a similar short latency has been described [19,20], but the mechanism by which it leads to histamine release is unknown; perhaps these patients also had IgM anti-IgE. A more precise understanding of the mechanism(s) of mast cell activation, which must account for the dependence on decreasing temperature, is not achieved by these present studies. Enhanced affinity of these antibodies for membrane bound IgE at 4°C as compared to 37°C may lead to aggregation of neighboring IgE molecules. Upon rewarming, activation of later membrane events may lead to mediator release. Attempts to support this proposal by performing the enzyme immunoassay at lower temperatures met with limited success as the binding increased for both patients and controls.

The finding of these autoantibodies in the syndrome of hypocomplementemic urticarial vasculitis is particularly interesting. This disorder is present with urticaria that is difficult to distinguish from idiopathic urticaria not associated with vasculitis [9]. However, circulating immune complexes and a Clq precipitin are invariably present [21] and may precipitate within the vasculature and surrounding tissues to initiate an inflammatory response. The role of basophils or mast cells in this process is not known; however, experimental models of serum sickness and nephritis suggest that the development of pathologic lesions is dependent on the release of vasopermeable substances [22]. Likewise, subcutaneous injection of histamine into uninvolved skin of patients with vasculitis may serve

Table II. Passive Transfer of Cold Reactivity

Patient	Enzyme Immunoassay		Passive Transfer		Basophil Histamine Release
	IgM Anti-IgE	IgG Anti-IgE	Ice Cube Challenge 5 h	48 h	
1	+4	—	1 + (1/3)	—	+
2	—	4+	4 + (2/2)	—	—
3	—	4+	4 + (2/2)	2+ (2/2)	—

to localize immune complexes and thereby induce a small-vessel vasculitis [23]. IgE autoantibodies might serve a similar function in urticarial vasculitis.

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