

Skin reactions to anti- γ E antibody in atopic, nonatopic, and immunologically deficient children and adults

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Guinea pig antibody to human γ E (anti- γ E) causes an immediate wheal and flare reaction, when small amounts are injected into human skin, considered to be mediated by antibody combining with γ E on skin cells. The minimum effective dose (MED) of anti- γ E causing a standard skin reaction was found to be related inversely to subjects' serum γ E concentrations. Nevertheless, comparison of MED determined on atopic and nonatopic persons failed to differentiate them reliably. Patients with general defects of immunoglobulin synthesis failed to react to anti- γ E except in very high doses, but patients with defects of only delayed-type reactivity reacted normally to anti- γ E.

Reaginic antibody activity in sera from atopic patients has been shown to be associated with a distinct class of immunoglobulins, IgE (γ E).¹ Specific antibody of the γ E class sensitizes human skin for the Prausnitz-Küstner (P-K) reaction²; nonantibody γ E blocks passive sensitization with reaginic antibody, whereas immunoglobulins of other classes fail to do so.^{3, 4} These findings strongly suggest that γ E has an affinity for cells involved with reaginic-type allergy in human beings.

In support of this, it has been found that guinea pig or rabbit antibody specific for human γ E (anti- γ E) causes an immediate wheal and flare when injected into normal human skin.⁵ The mechanism of this reversed-type reaction was considered to depend on combination of anti- γ E with normal γ E fixed to cells in the skin. The minimum effective dose (MED) of anti- γ E required to elicit such a reaction (in the range of 10^{-5} μ g AbN) was found to vary from person to person.

Johansson⁶ reported that serum γ E levels in adults with atopic asthma and hay fever tended to be higher, on the average, than in normal adults or in

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those with intrinsic asthma. The present studies were undertaken to evaluate the possibilities that an individual's MED of anti- γ E might reflect the serum or tissue γ E level and that this information could be useful in the clinical classification of patients with asthma.

MATERIALS AND METHODS

Human subjects for skin testing

Normal adults and children who denied any personal histories of atopic diseases (allergic rhinitis, asthma, or atopic dermatitis) were largely recruited from members of the staff of the Children's Asthma Research Institute and Hospital (CARIH) and from their families. Adults attending the allergy clinic of Colorado General Hospital for treatment of allergic rhinitis or asthma constituted the adult atopic group; all had significantly positive direct skin tests to common allergens. Children residing at CARIH for treatment of asthma made up the childhood asthmatic group. In addition to these groups, children and adults with various antibody-deficiency syndromes were also tested. These were patients in the wards and clinics of Colorado General Hospital, National Jewish Hospital, and Fitzsimmons General Army Hospital at Denver.

Skin test materials

The guinea pig antibody preparation reacting specifically with human γ E (anti- γ E) has been described previously.⁵ Guinea pigs were immunized with a reagin-rich fraction of serum from an allergic patient. The globulin fraction of the antiserum was absorbed with normal γ G and D-myeloma protein. The resultant preparation was monospecific for γ E in whole serum; it formed a precipitin band against a reagin-rich serum fraction identical to that formed by antiserum to an E-myeloma protein.⁵ The anti- γ E antibody concentration of the guinea pig antiserum was 60 μ g AbN per milliliter. Fresh dilutions were made on each day of testing from stock solutions containing 6 to 0.6 μ g AbN per milliliter, because higher dilutions lost potency in 1 to 2 days at 4° C.

Normal guinea pig serum was used as a control for skin testing. Serum of healthy guinea pigs, from blood obtained aseptically by cardiac puncture, was placed in sterile tubes in small aliquot parts and frozen at -70° C. until shortly before use.

Histamine diphosphate (Abbott Laboratories, No. Chicago) was purchased as a sterile solution and diluted in sterile saline for injection.

Allergic serum for Prausnitz-Küstner (P-K) sensitization was obtained from a subject allergic to ragweed. This serum has been used extensively in this laboratory with no untoward effects. The P-K titer of the serum for ragweed allergen was 1:4,000 to 1:16,000. Serum stored frozen in small aliquot parts was thawed shortly before use. Ragweed antigen extract for P-K testing was purchased from Center Laboratories. (Port Washington, N. Y.).

Skin tests

Proper concentrations of anti- γ E and histamine were controlled by testing a subject of known sensitivity on each day of testing. Dilutions in sterile saline

were made fresh each day. Subjects were generally tested between 2 and 5 P.M.; none was receiving antihistamines except in conjunction with experiments to be described, and none had received epinephrine or aminophylline for at least the previous 8 hours. Reagent dilutions were injected intracutaneously in 0.02 ml. through a No. 27 needle on the volar surface of forearms. Each subject received normal guinea pig serum (1:10,000), histamine (1 μ g per milliliter as histamine base), and various dilutions of anti- γ E. No delayed, adverse, or general reactions were found. The size of wheal and flare observed within 20 minutes of injection was recorded by tracing on a transparent sheet of hard plastic. The minimum effective dose (MED) of anti- γ E was that which gave a reaction most closely matching that caused by 0.02 μ g of histamine base. Six subjects showing positive skin reactions to normal guinea pig serum were rejected from the series, except for one asthmatic girl who had a small reaction to 1:10,000 dilutions of the guinea pig serum and a larger reaction to 1:30,000,000 dilutions of anti- γ E serum.

Prausnitz-Küstner (P-K) tests were performed on some subjects unreactive to ragweed extract. Allergic serum was diluted 1:500 to 1:16,000, and 0.05 ml. of the dilutions was injected into the volar surface of the forearms. After 48 hours the skin sites were challenged with 10 protein nitrogen units (PNU) of ragweed extract in 0.02 ml. of saline.

Serum γ E concentration measurements

Serum samples were shipped frozen to Drs. H. Bennich and S. G. O. Johansson at the University of Uppsala, Uppsala, Sweden, who measured the concentration of γ E by their radioimmunosorbent assay.⁷

EXPERIMENTAL RESULTS

Minimum effective dose of anti- γ E in normal subjects and persons with atopic diseases

As preliminary experiments, the reproducibility of determining the MED of anti- γ E was investigated by testing 21 subjects on subsequent afternoons. The results usually agreed, but in 6 subjects the tests differed by one threefold dilution (e.g., 3.6×10^{-5} to 1.2×10^{-5} μ g AbN). Results of tests performed on 9 subjects at 10 A.M. and 3 P.M. indicated lower sensitivity in the morning. All subsequent testing was done between 2 and 8 P.M. (usually between 2 and 5 P.M.).

The MED was determined on 22 "normal" adults (22 to 55 years old) and 5 "normal" children (5 to 12 years old) without personal histories of allergic rhinitis, asthma, or atopic dermatitis (Fig. 1). With only one exception, all minimum effect doses (MED) were between 3.6×10^{-4} and 1.2×10^{-5} μ g AbN.

Sixteen adults (21 to 83 years old) with allergic rhinitis or asthma and 145 children (6 to 17 years old) with asthma and related disorders were also tested. As shown in Fig. 1, substantial overlapping of MED values of the normal and atopic groups was found. Nevertheless 8 of the 145 children with asthma had a MED of 3.6×10^{-7} μ g AbN or less. These 8 cases included 2 of

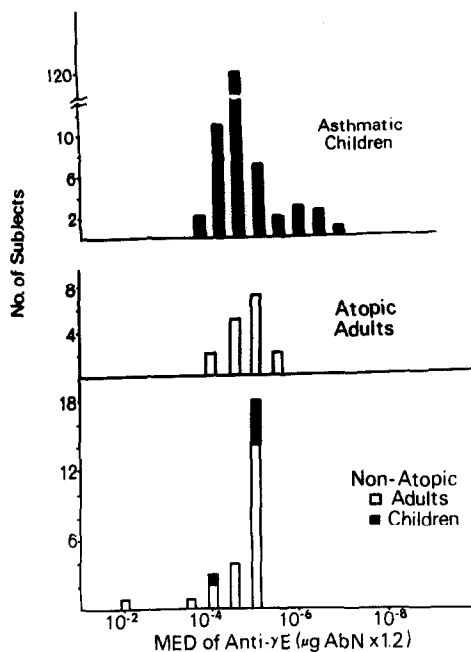


Fig. 1

The minimum effective dose (MED) of guinea pig antibody to human γ E causing a wheal-erythema reaction in a group of asthmatic children (*top*) was compared with that of a group of adults with atopic disorders (*middle*) and a group of adults and children denying such disorders (*bottom*). The scale indicating the number of asthmatic children is discontinuous.

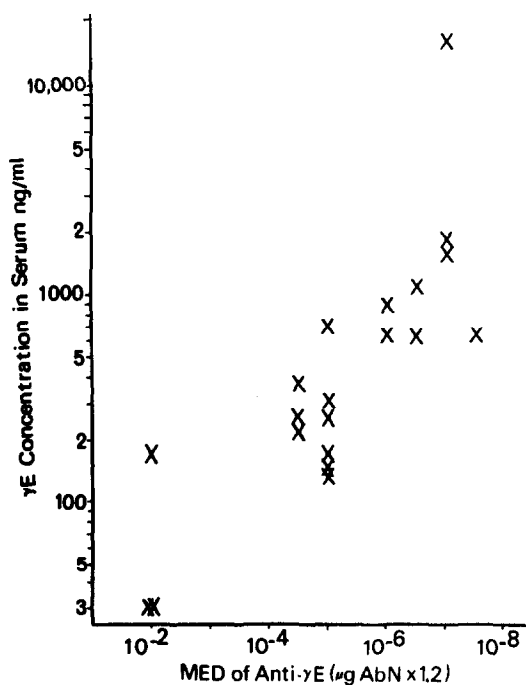


Fig. 2

The log of the MED of anti- γ E for 20 subjects, chosen to represent a wide range of values, is plotted against the log serum γ E concentration of each.

the 5 children with asthma and bronchiectasis and one girl who was subsequently found to be infested with *Ascaris*. All 8 patients had some positive immediate skin reactions to common allergens, such as pollens, molds, and epidermals.

The question was raised whether patients with intrinsic asthma could be differentiated from those with extrinsic asthma on the basis of their MED of anti- γ E. Nine children were selected who failed to react to intradermal skin tests with a wide variety of allergens considered to be likely precipitants of their asthma. These intrinsic patients were matched for age, sex, and race and duration and severity of asthma, with 10 children with extrinsic asthma having several significantly positive skin tests to common allergens. The MED of neither of these groups differed from that of normals.

The MED of anti- γ E was determined at least twice in each of 20 volunteers, and a serum sample from each was assayed for concentration of γ E.⁷ The results (Fig. 2) indicated that these subjects' serum γ E levels tended to

Table I. *Patients with antibody deficiencies*

Patient No.	Age (yrs.)	Sex	Diagnosis	Serum immuno-	
				mg./100	
				γ G	γ A
1	13	M	Hypogammaglobulinemia, sex-linked	130	< 2
2	18	M	Hypogammaglobulinemia, sex-linked	113	< 6
3	15	M	Hypogammaglobulinemia, primary acquired	81	< 2
4	14	M	Hypogammaglobulinemia, primary acquired	78	< 2
5	51	M	Hypogammaglobulinemia, primary acquired	207	13
6	18	M	Hypogammaglobulinemia, primary acquired	38	< 2
7	9	F	Hypogammaglobulinemia, primary acquired	325	28
8	3	F	Thymic aplasia (familial)	1035	57
9	5	M	Wiscott-Aldrich syndrome	1200	450
10	10	M	Chronic cutaneous granulomatous candidiasis	810	2
11	12	M	Chronic cutaneous granulomatous candidiasis	1152	151
12	6	F	Intestinal lymphangi- ectasia with protein- losing syndrome	158	31

*MED = Minimum effective dose for skin reaction.

†ND = not done.

be inversely related to their MED of anti- γ E, although the correlation was obviously imperfect.

Skin tests in patients with antibody-deficiency syndromes

Twelve patients with various forms of immunologic defects were tested (Table I). Seven patients (Nos. 1 to 7) had primary defects of immunoglobulin synthesis of both congenital and late-onset types. The γ E concentration in sera from 2 of these patients was found to be as low as that in umbilical cord serum.⁸ These patients either failed to react to anti- γ E injections or reacted to such high doses that the specificity of the reaction was doubtful. Nevertheless, it was possible to sensitize the skin of Patient No. 1 for a P-K reaction to ragweed allergen. The maximal dilution of allergic serum sufficient to sensitize his skin was 1:2,000, which was not significantly different from that of normal persons.

Four patients (No. 8 to 11) were deficient primarily in the delayed-type

<i>globulins</i>		<i>MED* of anti-γE (μg AbN)</i>	<i>Delayed-type reactivity</i>	<i>Comment</i>
<i>ml.</i>	γ E (ng./ml.)			
γ M				
26	ND†	1.2×10^{-2}	OK	Seasonal rhinitis
< 8	ND	$> 1.2 \times 10^{-2}$	OK	—
15	ND	1.2×10^{-2}	OK	Angioedema after penicillin
< 5	30	1.2×10^{-2}	OK	Family history of asthma and hay fever
7	ND	3.6×10^{-3}	OK	Asthma in childhood
296	ND	1.2×10^{-2}	OK	Idiopathic hives, anaphylac- toid reaction to γ G ⁹
30	30	1.2×10^{-2}	Defective	Few plasma cells lymphopenic
204	ND	1.2×10^{-5}	Defective	Family history of hay fever
100	ND	1.2×10^{-4}	Defective	
41	ND	1.2×10^{-5}	Defective	Brother of No. 11
54	ND	1.2×10^{-3}	Defective	—
63	ND	1.2×10^{-3}	OK	—

antibody system. Although Patient No. 10 also had low serum and salivary γ A, all 4 reacted normally to anti- γ E.

One girl (No. 12), although able to synthesize immunoglobulin, was hypogammaglobulinemic because of protein loss due to intestinal lymphangiectasia, as shown by jejunal biopsy. Abnormally high serum protein concentrations were found in small-bowel fluids, serum proteins in the blood were generally depressed, and protein catabolic rates were increased. The MED of anti- γ E was $1.2 \times 10^{-3} \mu\text{g}$ AbN, suggesting that the protein losses included γ E.

Three hypogammaglobulinemic patients with reduced reactivity to anti- γ E had histories of allergy-like disorders as has been previously described.⁹ Patient No. 1 was suspected of having seasonal allergic rhinitis; skin tests to many inhalent and food allergens were negative, and no allergen was identified historically. Patient No. 3 had one episode of angioneurotic edema following a penicillin injection; his skin test to penicillin G was negative. Patient No. 6 has had idiopathic urticaria and, at another time, an anaphylactoid reaction to a γ -globulin injection. His serum contained an antibody to aggregated human γ G¹⁰ but did not sensitize normal skin for a P-K reaction to this antigen. Although it is very possible that some of these reactions were not truly allergic (not mediated by antigen-antibody combination) or may have been dependent on antibody of some other class, it should be noted that even the reduced γ E concentrations found were far in excess of the concentration of γ E antibody needed to sensitize skin for a positive P-K reaction.¹¹

Effect of drugs on skin reaction to anti- γ E

No difference in sensitivity could be found between asthmatic children receiving adrenal corticosteroids and those not on such treatment. The same was true for ephedrine (25 mg., 4 times a day). However, an antihistamine (chlorpheniramine) was found to depress reactivity to anti- γ E, histamine, and ragweed antigen in parallel. Three subjects were tested with anti- γ E, histamine, and ragweed extract. One subject reacted directly to ragweed; skin reactivity in the other 2 was induced by passive transfer with allergic serum. Tests were performed before the first dose and 2 hours after the last dose of chlorpheniramine (4 mg. of Chlor-Trimeton) orally 4 times for one day. After the antihistamine, the first subject required 10 times as much histamine, ragweed extract, and anti- γ E to give the same reactions as before treatment. The other 2 subjects required 3 times as much anti- γ E after treatment, and their P-K titers dropped from 1:4,000 before to 1:2,000 and 1:1,000 after chlorpheniramine.

DISCUSSION

In a previous article⁵ it was shown that wheal-erythema reactions in human skin could be induced by anti- γ E antibodies but not by antibodies specific for the other classes of immunoglobulin. In view of evidence that γ E-immunoglobulin is a carrier of reaginic antibody¹ and has affinity for cells involved in reaginic-type allergic reactions,³ the skin response to anti- γ E was considered to be a reversed-type allergic reaction induced by combination of the antibodies with cell-bound normal γ E. This idea found support in subsequent studies¹² which

demonstrated that anti- γ E releases histamine from human leukocytes in vitro. With the use of that system, the concentration of anti- γ E required to release 50 per cent of cell histamine was decreased if the cells were first passively sensitized in vitro by incubation with a γ E-rich serum. In a similar way, the minimum dose of anti- γ E required for a positive skin reaction was decreased by previous injection of γ E-rich serum into the skin site.

These findings strongly suggest that the sensitivity of skin to anti- γ E is related to the local amount of cell-bound γ E. If such cell-bound γ E is in equilibrium with the protein in tissue fluids or serum, one might expect an inverse relation between an individual's MED and serum γ E concentration. Results from 20 subjects (Fig. 2) did show a roughly inverse relation between these variables. In fact, the patient having the highest serum γ E level was most sensitive to anti- γ E, reacting to 0.12 pg. (10^{-12} Gm.) AbN, whereas a hypogammaglobulinemic patient failed to react to even 12,000 pg. AbN of anti- γ E injected intradermally. The fact that the correlation was imperfect is not especially surprising. The sensitivity of the reaction is probably determined by biochemical processes leading to the release of chemical mediators, as well as by the amount of cell-bound γ E, and a degree of individual variation can be expected both in the efficiency of the former mechanism and in the ratio of serum to cell-bound γ E.

The present studies were undertaken with the expectation that a person's MED of anti- γ E would help to classify him as atopic or nonatopic. Johansson⁶ has reported that 10 of 16 adults with extrinsic asthma had serum γ E levels above 700 ng. per milliliter, a value exceeded by only one of 22 intrinsic asthmatic patients and 3 of 58 healthy blood donors. From Fig. 2 it can be seen that a serum γ E concentration of 700 ng. per milliliter would correspond to an MED of approximately 10^{-6} μ g AbN. The vast majority of the adult atopic and childhood asthmatic subjects failed to react to this dose, apparently signifying that our atopic patients differed from those of Johansson. Even when sera from our extrinsic asthmatic patients who were highly sensitive to anti- γ E were assayed for γ E levels, they tended to be lower than those of his 10 extrinsic asthmatic subjects.

Although it is possible that a larger series might demonstrate a statistical difference between the average MED of normal persons and extrinsic asthmatic subjects, the available data are adequate to show that an individual's MED of anti- γ E does not reliably categorize his status as regards atopic disease. A substantial number of patients with extrinsic asthma and hay fever have a MED in the normal range; measurement of serum γ E levels in some of these patients also showed normal γ E concentrations. Such results are in accord with the well-known fact that the severity of asthma often correlates poorly, if at all, with the intensity of skin reactions to allergens or with the P-K titer of a patient's serum.

The essential characteristic of the atopic state is not the presence in the serum of γ E, for it is a normal immunoglobulin, nor is it the ability to develop a wheal-erythema reaction mediated by reaginic antibody, as could have been inferred from the fact that allergic serum transfers reactivity to normal skin. It seems that the differentiating characteristics of the atopic condition might

profitably be sought in quantitative studies of factors determining the synthesis of specific γ E antibodies, especially as they relate to production of antibodies of other immunoglobulin classes. Other important areas for study include the reactivity of target organs to chemical mediators¹⁴ and the biochemical steps leading to the release and generation of such mediators. As regards the last area, Lichtenstein and Norman¹⁵ have found that the sensitivity of leukocytes from allergic patients to allergen-induced histamine release may be decreased by immunotherapy and that such change may be independent of serum antibodies. This has clinical significance in that the sensitivity of leukocytes to allergen correlates with the clinical severity of hay fever.¹⁶ Moreover, studies in our laboratories¹² have shown that leukocytes from atopic patients release histamine at lower concentrations of anti- γ E than are required for leukocytes from normal persons, even when serum levels of γ E are comparable.

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