

Late cutaneous allergic responses in isolated IgE-dependent reactions

J. Dolovich,* F. E. Hargreave, R. Chalmers, K. J. Shier, J. Gauldie,
and J. Bienenstock *Hamilton, Ontario, Canada*

Relationships between IgE-dependent reactions and late cutaneous allergic responses were examined. Heterologous whole antihuman IgE or its pepsin digest F(ab')₂ product injected intracutaneously produced "immediate" wheal and flare responses followed by late responses. Prausnitz-Küstner reactions were similar in appearance. The late responses were studied morphologically by conventional microscopy and by direct immunofluorescent techniques. The results support the thesis that in man, late cutaneous allergic responses can occur in reactions with an isolated involvement of IgE. There is a discussion of the possible relevance of these observations to antigen-induced allergic reactions in skin and in the respiratory tract.

The type I reaction¹ in human skin takes the form of a wheal and flare maximal at about 10 minutes.² It is typically initiated by the interaction of antigen with reaginic antibody in sensitized tissues. Reaginic or long-latency antibody activity³ in man is largely or exclusively a function of antibody of the IgE class.⁴ Short-latency antibodies of the IgG class⁵ may also be involved in type I reactions. Type I allergic reactions can also be initiated by the addition of heterologous antihuman IgE to appropriate human cells or tissues that contain IgE. The intracutaneous injection of antiserum to human IgE produces the so-called reversed type reaginic reaction.^{6, 7}

Careful observations of the progression of individual allergic reactions in man have revealed durations of reactions not previously appreciated. The intracutaneous injection of *Aspergillus* antigen in persons with allergic bronchopulmonary aspergillosis (ABA) produces "immediate" wheal and flare reactions followed by responses that are maximal at about 6 to 12 hours and are termed "late" responses.⁸⁻¹⁰ These so-called "dual" reactions, with immediate and late components, have also been observed in tests with avian sera in persons with extrinsic allergic alveolitis (EAA) from exposure to dusts of pigeons and budgerigars.^{9, 11} The immediate reactions in these situations may not be caused by the same mechanisms in all instances; serum IgE antibodies to the offending antigens were, for example, not demonstrated in a study of EAA due to ex-

From the Departments of Pediatrics, Medicine, and Pathology, St. Joseph's Hospital and McMaster University.

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Reprint requests to: Dr. J. Dolovich, Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada.

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posure to avian proteins.¹² The biphasic property of the dual reaction suggests the possible participation of multiple processes perhaps including multiple types of antibodies. The late reactions of ABA and EAA have been found to correlate closely with serum precipitins, generally antibodies of the IgG and IgM classes. There are edema, infiltration, and perivascular cuffing, chiefly with neutrophilic leukocytes and mononuclear cells and some eosinophils. Deposition of IgG, IgM, and β_1C within the vascular endothelium and in the cytoplasm of the perivascular inflammatory cells has been observed.⁹ As a result of findings such as these, the late components of allergic reactions have come to be considered synonymous with Arthus or type III reactions.

Recently, dual skin reactions have been observed in some patients with uncomplicated extrinsic rhinitis or asthma from exposure to airborne *Bacillus subtilis* enzyme preparations,¹³ grass pollen,¹⁴ and ragweed pollen.¹⁵ In these actively sensitized subjects, serum precipitins can generally not be detected by usual techniques; nevertheless, serum antibodies of multiple immunoglobulin classes are commonly present, thereby providing a potential basis for mixed types of reactions. The deposition of β_1C in late responses of skin tests of grass pollen-sensitive subjects has suggested a combined type I and type III reaction.¹⁴ However, reactions with gross characteristics of dual responses at the sites of ragweed antigen E-induced Prausnitz-Küstner (PK) tests¹³ have been considered to be reagin-dependent and largely or exclusively a function of antibodies of the IgE class.

The present investigations were designed to examine the gross, microscopic, and immunofluorescent appearances of cutaneous reactions in human skin in which there is an apparent isolated involvement of IgE and to compare them with antigen-induced reactions in naturally sensitized subjects. The reactions examined included passive transfer reactions and reversed type reaginic reactions induced by heterologous antiserum to human IgE and an IgG F(ab')₂ preparation from this antiserum.

MATERIALS AND METHODS

Subjects

Tests were performed on 12 subjects. Their ages, sex, and atopic status are presented in Table I. Eight were nonatopic and had no allergic respiratory disease. Four were atopic and had asthma or rhinitis.

Intracutaneous tests

These were performed on the forearm by the injection of approximately 0.02 ml. of the test solution by one of us (R. C.). The sites were examined at 10 minutes and at intervals up to 48 hours by a second observer with no knowledge of the distribution of the different forms of challenge. The diameters of the reactions were measured in 2 perpendicular directions, and characteristics at different times were noted.

Test materials

The following materials were used for skin tests:

1. Antiserum to PS human myeloma IgE was prepared in sheep. This was absorbed with an agarose-insolubilized immunoabsorbent of normal human serum (NHS). The antiserum produced a single line of precipitation in double-diffusion reactions with PS IgE

TABLE I. Patient details

Patient	Sex	Age (yr.)	History of atopic disease	Positive allergy skin tests*
1	F.	47	Neg.	0
2	F.	29	Neg.	0
3	F.	41	Neg.	0
4	F.	27	Neg.	0
5	M.	32	Neg.	0
6	M.	33	Neg.	0
7	M.	31	Neg.	0
8	F.	29	Neg.	0
9	M.	25	R.	0
10	M.	31	R.A.	3
11	M.	24	R.A.	6
12	M.	27	E.A.	10

E. = Eczema. A. = Asthma. R. = Rhinitis.

*Number positive in a series of skin tests by the prick test method with 20 common allergens.

myeloma protein, but there was no visible precipitate in reactions with NHS. Radio-immunodiffusion tests, by a 2 step method previously described,¹⁶ with the sera of subjects sensitive to ragweed and *Aspergillus* antigens, demonstrated that the antiserum reacted with nonmyeloma IgE. In tests of these human sera, dissociations in classes of antibodies demonstrable provided further evidence of the monospecific property of the antiserum to human IgE. The antiserum induced a wheal and flare response in the skin of normal human subjects in dilutions up to approximately 1:2,500; it was used in a 1:10 dilution.

2. Normal sheep serum (NSS) diluted 1:10 was used.

3. The absorbed sheep antihuman IgE was precipitated with 33 per cent $(\text{NH}_4)_2\text{SO}_4$, redissolved and dialyzed. It was then digested with pepsin and the IgG F(ab')₂ was purified by Sephadex G-100 gel filtration. This preparation gave a symmetric 5S peak in the analytic ultracentrifuge with no evidence of heavier material. It was then shown to precipitate IgE. This preparation appeared to be slightly less potent than the whole antihuman IgE in the induction of wheal and flare responses and was used in a 1:3 dilution.

4. An F(ab')₂ preparation comparable to that described above was produced from NSS and used in 1:3 dilution.

5. For PK tests, serum with reaginic antibodies to *B. subtilis* alpha amylase was obtained from a detergent industry worker. Tests of the serum for Australia antigen were negative. The donor had no history of jaundice and no biochemical evidence of liver disease, and the serum has been used in PK tests for more than 2 years with no clinical or biochemical evidence of hepatitis in recipients. The serum, diluted 1:3, was injected intracutaneously in 0.1 ml. volume to produce sensitization. After a 3 day latency, the sites were challenged with antigen.

6. Crystalline *B. subtilis* alpha amylase was obtained from Nagase and Co. Ltd., Omaha, Amagasaki, Japan. A 100 μg per milliliter solution was used for intracutaneous challenge of passively sensitized and control skin sites.

7. A whole ragweed pollen extract (30,000 PNU per milliliter) obtained from Omega Laboratories, Don Mills, Ontario, Canada, was dialyzed and used in a 1:10 dilution.

8. Histamine acid phosphate (The British Drug Houses [Canada] Ltd., Toronto, Ontario, Canada) was used in a concentration of 0.36 mg. of histamine base per milliliter.

8. A sample of compound 48/80, provided as a gift by Burroughs Wellcome & Co. (Canada), Ltd., was used in a concentration of 37.5 mg. per milliliter.

The reagents were sterilized by passage through a millipore filter and stored at 4° C. until used.

Skin biopsies

Skin biopsies were taken at about 6 hours after the onset of cutaneous reactions. The particular reactions biopsied are indicated in Table II. Specimens for histologic examination

TABLE II. Diameters in millimeters of skin test reactions

Subject	Anti-IgE		F(ab') ₂ of anti-IgE		Prausnitz-Küstner		Ragweed pollen antigen		NSS	F(ab') ₂ of NSS	Compound 48/80	Histamine
	10 min.	6-12 hr.	10 min.	6-12 hr.	10 min.	6-12 hr.	10 min.	6-12 hr.	10 min.	10 min.	10 min.	10 min.
1	9	53	11	50	—	—	—	—	5	5	8	11
2	6	66	6	0	—	—	—	—	5	5	6	6
3	6	38	7	33	—	—	—	—	6	—	9	11
4	8	61	10	80	—	—	—	—	4	4	10	9
5	—	—	8	40*	—	—	—	—	4	4	—	—
6	10	70*	—	—	—	—	—	—	4	—	—	—
7	12	68	17	73*	15	36*	—	—	4	5	14	5
8	6	37	8	33	8	50	—	—	4	5	6	6
9	8	48*	8	34*	—	—	—	—	0	5	—	—
10	8	40	7	23	—	—	13	64	5	—	10	8
11	—	—	—	—	—	—	15	45*	—	—	—	—
12	—	—	—	—	—	—	6	30*	—	—	—	—

— = Not done.

Values for 10 minute reactions represent mean diameters of wheals. Values for 6 to 12 hour reactions represent mean diameters of swellings.

*Biopsies were performed.

were stained with hematoxylin and eosin, with orcein-Giemsa and toluidine blue stains for mast cell examination, and with the M.S.B. method of Lendrum for the demonstration of fibrin deposition.

Portions of the biopsies were processed in 10 per cent buffered formalin or, in some instances, by the Sainte-Marie method¹⁷ for immunofluorescent studies. Tests were performed by the direct method with monospecific fluorescein-labeled sheep antisera to human IgG, IgA, IgM, and β_2 C.

Serologic tests

Tests for serum precipitins to ragweed pollen antigen were performed by double-diffusion in agar.

RESULTS

Gross appearances of cutaneous reactions

The sizes of cutaneous reactions to the various reagents are described in Table II.

There were immediate and late components in the cutaneous reactions produced by sheep antiserum to human IgE and the F(ab')₂ preparation of anti-IgE as well as *B. subtilis* alpha amylase in passively sensitized sites. The gross characteristics resembled ragweed antigen-induced reaction in Subjects 10, 11, and 12 who were naturally sensitive. The reactions began with a wheal and flare. Then, while the flare decreased, the swelling at the site of the wheal persisted. At 2 to 4 hours the swelling began to increase in diameter, and this continued over a number of hours. It was soft, ill defined, and variably erythematous, pruritic, and slightly tender. It then began to subside and completely disappeared within 24 to 48 hours.

In contrast, the control tests with normal sheep serum (NSS), the F(ab')₂ preparation of NSS and *B. subtilis* alpha amylase, produced only small red papules less than 6 mm. in diameter within 15 minutes after injection; these

TABLE III. Microscopic appearances of skin biopsies

Subjects	Anti-IgE		F(ab') ₂ of anti-IgE			Prausnitz-Küstner	Ragweed antigen	
	6	9	5	7	9	7	11	12
Edema	3+	2+	2+	2+	2+	2+	3+	4+
Mast cell degranulation	1+	2+	2+	2+	1+	2+	3+	3+
Eosinophils	3+	1+	2+	2+	2+	3+	3+	5+
Neutrophils	1+	1+	1+	1+	1+	±	3+	2+
Mononuclear cells	1+	1+	1+	1+	1+	-	±	±

responses cleared over a period of 2 to 3 hours. In addition, at sites injected with NSS, in Subjects 2, 3, and 6, there were short-lived wheal and flare reactions with an onset at 6.5, 8.5, and 72 hours, respectively. These responses were unexplained and were not considered comparable to the reactions produced by antiserum to IgE.

Tests with histamine and compound 48/80 caused wheal and flare reactions reaching a maximum within 10 or 15 minutes. The reactions caused by histamine cleared completely within 2 hours. In the case of compound 48/80, the wheal subsided rapidly; the swelling and erythema cleared more slowly, but at 4 hours there was only a patch of erythema that measured less than 1 cm. in diameter.

Microscopic and immunofluorescent studies of skin biopsy specimens

Table III describes the histologic features of the skin biopsies. The various indices of response are arbitrarily graded from absent (-) to maximal (5+). The appearances were quite similar in all specimens. They consisted of edema, mast cell degranulation of varying intensities, and perivascular infiltration. The predominant cell type of the perivascular infiltration was the eosinophil. Neutrophils were scanty but possibly more numerous in the antigen-induced reactions of the ragweed-sensitive subjects. There was no evidence of intravascular thrombi, necrosis of blood vessels, or fibrin deposition. In sites challenged with anti-IgE or F(ab')₂ of anti-IgE, there was also a mild admixture of mononuclear cells including lymphocytes and monocytes but no plasma cells.

Immunofluorescent studies of tissues removed for biopsy failed to reveal any deposition of IgG, IgM, IgA, or β_2 C.

DISCUSSION

The gross appearances of skin reactions induced by sheep antihuman IgE and its pepsin digestion F(ab')₂ product and by *B. subtilis* antigen in PK tests resembled ragweed antigen-induced reactions in actively sensitized subjects. There were the features of "dual" responses. No such responses were produced by normal sheep serum (NSS), a pepsin digestion product of NSS, or by *B. subtilis* antigen in nonsensitized sites. Immediate wheal and flare responses in reversed type and PK reactions were comparable in size to those of histamine and compound 48/80-induced reactions; however, the latter subsided quite

rapidly. Thus the observed late responses of reversed type and PK reactions were not a simple function of the sizes of immediate wheal and flare responses.

Anti-IgE has a unique capacity among antisera to immunoglobulins in the induction of wheal and flare responses and histamine release from leukocytes. These reactions of the reversed type appear to be entirely comparable to antigen-induced responses.^{6, 7, 18} The observed occurrence of late responses in reversed type reactions provides a potentially useful procedure for the examination in normal as well as in allergic subjects of conditions that influence late responses. Moreover, the immunologic reactants involved are apparently determined by the specificity of the antiserum used; as a result, these are probably more representative of isolated types of reactions than antigen-induced reactions in actively sensitized individuals. Procedures were designed to minimize the possibility of involvement of immunoglobulins other than IgE. The anti-human IgE was absorbed with an insolubilized NHS antigen to avoid the possible formation of soluble complexes. Antibodies to idiotypic determinants of sheep immunoglobulin could represent an alternate form of reactivity, however, this is unlikely to occur in all subjects tested. The pepsin digestion of a globulin preparation of the anti-IgE and purification by gel filtration of the IgG F(ab')₂ would be expected to reduce the possible reactivity of sheep serum proteins with antibodies of the recipients. Reversed type reactions were compared with reagin-dependent PK reactions considered to be largely or exclusively a function of antibody of the IgE class.¹⁹

It has been generally considered that the late allergic responses following immediate IgE-dependent reactions involve immune complex injury or so-called type III reactions. There is an observed tendency of vasoactive amines derived from IgE-dependent reactions to contribute to the deposition of immune complexes.²⁰ There is a well-defined participation of complement and neutrophils in reactions of this type. The observed deposition of IgG and β_1C in some late cutaneous responses in man in ABA and EAA supports the concept that immediate and late allergic responses may have separate immunologic derivations. Reversed type reaginic reactions induced by sheep antihuman IgE could potentially involve processes of this type through the interaction of C1 of the recipient with injected antibody to IgE. However, the pepsin-digested product of IgG retained the capacity to induce late allergic responses despite the inability of this type of preparation to activate more than trace quantities of hemolytic complement.²¹

As an additional possibility, the F(ab')₂ of IgG could be operative through the bypass activation sequence²² that represents an alternative process of complement activation. However, no support for this process was obtained. No deposition of immunoglobulins or β_1C could be demonstrated in biopsies of late responses. In addition, the infiltrations were predominantly eosinophilic. The results suggest that the late responses of reversed type reactions did not depend on the involvement of the complement system, at least in a manner corresponding to presently recognized processes. This does not contradict reports that demonstrate the involvement of this system in late responses; the implication is that the described involvement is not obligatory.

The simplest explanation of the late responses observed in reversed type and PK reactions would directly relate the tissue changes with IgE. Potentially, there could be a local persistence of immunologic reactants producing prolonged stimulation of mast cells at the reaction site. The acute reaction might also result in the liberation or production of undefined mediators that have prolonged effects. Alternately, the late responses might be the result of a participation of infiltrating cells. It is an interesting possibility that eosinophilic leukocytes could be involved in the pathogenesis of reactions of this type in a manner analogous to the involvement of neutrophilic leukocytes in immune complex reactions. Whatever the mechanism of amplification and prolongation involved in late responses, the threshold requirement for reacting antigen or anti-IgE is greater than for the preceding wheal and flare response. The intracutaneous injection of ten- to twenty-fold more anti-human IgE and its F(ab')₂ product was required for well-defined late responses than for wheal and flare responses.

Late responses in reversed type and PK reactions were grossly similar to antigen-induced reactions in sensitive individuals. Microscopic examination indicated that the latter reactions had greater numbers of neutrophilic leukocytes and fewer mononuclear cells. There was not a predominance of mononuclear cells or a demonstrable deposition of β_1C as have been reported in antigen-induced late reactions in patients with ABA and EAA and in allergy to grass pollen. The individuals actively sensitized to ragweed antigen would be expected to develop antigen-induced reactions similar to those of grass pollen-allergic subjects by Taylor and Shivalkar.¹⁴ The apparent differences in microscopic and immunofluorescent findings in the late responses may reflect a selection process. Late responses were observed in about 30 per cent of skin test reactions in the grass pollen-allergic subjects, and biopsies were done on some of these. The conditions of production of the reactions may somehow have been appropriate for the induction of late responses in instances in which there was a predisposition for β_1C involvement.

Due to the easy accessibility, it is allergic reactions in skin that have been examined. However, the interest in these reactions derives in large measure from the apparent analogy to antigen-induced reactions of the respiratory tract. Allergen inhalation tests produce dual asthmatic responses in ABA and late peripheral respiratory responses in EAA. The characteristics of the respiratory responses and the fever and neutrophilic leukocytosis have been considered to indicate that the antigen challenges reproduce many of the features of the diseases. Recently, it has become evident that antigen-induced dual asthmatic responses are usual in persons with uncomplicated asthma from sensitivity to common allergens including house dust,^{23, 24} grass pollen,²⁵ ragweed pollen,¹⁵ and avian antigens.²⁶ Apparently, no fever or neutrophilic leukocytosis has been observed. The processes involved in the airway responses in these instances might, in large measure, be analogous to the cutaneous responses with isolated IgE dependence described here. It is evident from these considerations that the designation "late cutaneous allergic response" or "late respiratory allergic response" may be inadequate to differentiate the patterns of responses that can be encountered.

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