

Immunochemical properties of some atopic allergens

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Many substances can act as antigens or allergens to induce immediate or delayed hypersensitivity reactions in animals and man. Antigens which induce immediate hypersensitivity reactions mediated by specific IgE antibodies are designated as atopic allergens. In this presentation I will review some of the known immunochemical properties of atopic allergens.

Many environmental sources of atopic allergens are of clinical interest. Several of these common sources have been studied for their allergenic components.¹⁻⁴ These sources include inhalants such as pollens, animal danders, and mites, injected antigens such as from bee and vespid venoms, and ingested foods such as fish, egg, and milk. In all cases cited, allergenic activity is found to be associated with the protein-rich fractions.

Allergens from each source can be divided into major and minor ones, based on their relative allergenic activities. Major allergens are highly active in the majority of allergic individuals tested, while minor allergens are poorly active. However, minor allergens can be as active as, or more so than, major allergens in some selected individuals. This variation in the individual response to different allergens is believed to be under genetic control.³

Major allergens are usually in the molecular size range of 20,000 to 40,000 daltons, while the size of minor allergens is highly variable from a few thousand to about 70,000 daltons. Second, major allergens are usually the more abundant proteins in the extract than minor allergens. A third common observation is that the allergenic activity of allergens is markedly reduced on denaturation. This indicates that most, if not all, of the antigenic determinants reacting

with specific IgE antibodies are dependent on the native conformation of the molecule. Apart from these features of molecular size distribution, relative abundance and conformational dependence, the allergens from different sources do not share other discernible, biochemical features, such as isoelectric point, carbohydrate content, amino acid composition or amino acid sequence, and enzyme activity. The foregoing generalizations can be illustrated with the findings made on allergens from ragweed pollen and honeybee and vespid venoms.

Ragweed pollen contains at least 10 antigenic proteins which are detectable with animal antisera. Six of these antigens have been purified. Their physiochemical properties are shown in Table I. AgE and AgK are acidic proteins of about 38,000 daltons.⁵⁻⁷ AgRa3, AgRa4 (BPA-R), and AgRa5, and cytochrome c are basic proteins of 11,000, 23,000, 4,970, and 13,000 daltons, respectively.⁸⁻¹² AgRa3 and AgRa4 are glycoproteins; the others are not. These 6 proteins together represent about 11% of the pollen proteins, with AgE and AgK as the most abundant components. With the exception of cytochrome c, the biochemical function of these proteins is not known.

By skin tests and histamine-release assays, AgE has been shown to be 10 to 100 times more active than the pollen protein mixture in about 95% of the patients tested,⁵ and AgK was about half as active as AgE.⁶ In contrast, other ragweed antigens are highly allergenic in only a fraction of the subjects tested. This can be illustrated by a recent report¹³ in which solid-phase ragweed antigens were used to determine the frequency of elevated antigen-specific IgE antibody levels in ragweed-sensitive individuals. A clear majority of the individuals (97% and 88%) showed elevated levels of IgE antibodies specific for AgE and AgK, while a smaller fraction (51%, 28%, and 17%) showed elevated levels of IgE antibodies specific for AgRa3, AgRa4, and AgRa5, respectively. In one survey of 31 ragweed-sensitive sera,¹⁴ antigen E-specific IgE antibody was found to account for 0.5% to 14% of total IgE with an arithmetic mean of 4.5%.

Honeybee venom is a simpler mixture of peptides and proteins than is ragweed pollen. Three of its components are known to be allergenic in man.¹⁵ They are

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TABLE I. Physicochemical properties of some atopic allergens

Antigen	MW	Isoelectric point (pH)	Nitrogen content (%)	Carbohydrate content (%)	Percent of proteins	References
<i>Ragweed pollen*</i>						
AgE	37,800	5.0	17.1	<0.5	6	5, 7
AgK	38,000	5.9	16.6	<0.5	3	6, 7
AgRa 3	12,100	8.6		8.0	0.4	8, 11
AgRa 4	28,000	8			0.9	9, 11
AgRa 5	5,000	9.5		0	0.1	10, 11
Cytochrome c	13,000	>10			0.1	12
<i>Honeybee venom</i>						
Phospholipase A ₂	15,800	Basic			17	15, 19
Hyaluronidase	50,000	Basic			3	15, 19
Melittin	2,800	Basic		0	71	15, 19
<i>Vespid venoms†</i>						
Antigen 5	25,000	Basic			15	16
Phospholipase	35,000	Basic			0.4	16
Hyaluronidase	45,000	Basic			2	16

*Some of the antigens are isolated in multiple isoelectric forms; the isoelectric point given is for the predominant form of the antigen of interest. Antigen Ra 4 is also known as antigen BPA-R.

†Vespid venoms from white-faced hornet, yellow hornet, and yellow jacket were studied and found to have very similar protein compositions.

TABLE II. Relative allergenic activities of honeybee venom components*

Patient No.	Hyaluronidase	Phospholipase	Melittin
1	0.6	12	0.006
2	1	12	0.1
3	1	5	0.005
4	1	3	
5	5	10	0.33
6	4	6	0.04
7	90	0.5	0.09

*Data taken from reference 15. Allergenic activities were tested by histamine release assays with leukocytes from sensitive patients. Activities given are relative to those of whole venom.

hyaluronidase, phospholipase A₂ and melittin of 50,000, 15,800, and 2,840 daltons, respectively, (Table I). These account for 91% of the weight of venom protein. Two of these components have enzymic activities as indicated by their names, while melittin has hemolytic activity. Histamine-release tests with sensitive leukocytes showed these 3 components to have variable activities, as shown in Table II. Phospholipase is clearly the major allergen, since it is about 5 times more active than venom in 6 of the 7 patients tested. Hyaluronidase is more active than venom in only 3 of the 7 tested. Melittin is a weak minor allergen with about 1/20 of the activity of venom. The widely variable responses seen in different individuals to different antigens are apparent upon comparing Patients 1, 5, and 7.

The correlation of allergenic activity of an antigen with its relative abundance can be further demonstrated by the comparative findings on honeybee and vespid venoms. White-faced hornet, yellow hornet, and yellow jacket venoms have very similar protein compositions when analyzed by gel chromatography and electrophoresis.¹⁶ All 3 vespid venoms contain hyaluronidase of 45,000 and phospholipase of 35,000 daltons. In contrast to honeybee venom, these 2 enzymes are minor protein components of vespid venoms. The major component of vespid venom is a basic protein of about 25,000 daltons, designated as antigen 5, of unknown biochemical function (Table I). Antigen 5 is in fact the major allergen of vespid venoms. Another point of interest is the partial antigenic cross-reaction of the 3 respective antigens of the 3 vespid venoms. However, there is no cross-reaction of any of the components of vespid venom with those of bee venom.

The amino acid sequences of at least 5 allergens are known. These allergens are ragweed AgRa3¹⁷ and AgRa5,¹⁸ bee venom phospholipase and melittin,¹⁹ and codfish allergen M.²⁰ The sequence data do not reveal any unusual features. At present we have no data on the structure and size of the antigenic determinants of any of these allergens reacting with their specific IgE or IgG antibodies. Attempts to study the determinants of ragweed AgE,²¹ AgRa3, and AgRa5²² have been hampered by the finding of strong conformational dependence of these allergens for their activities.

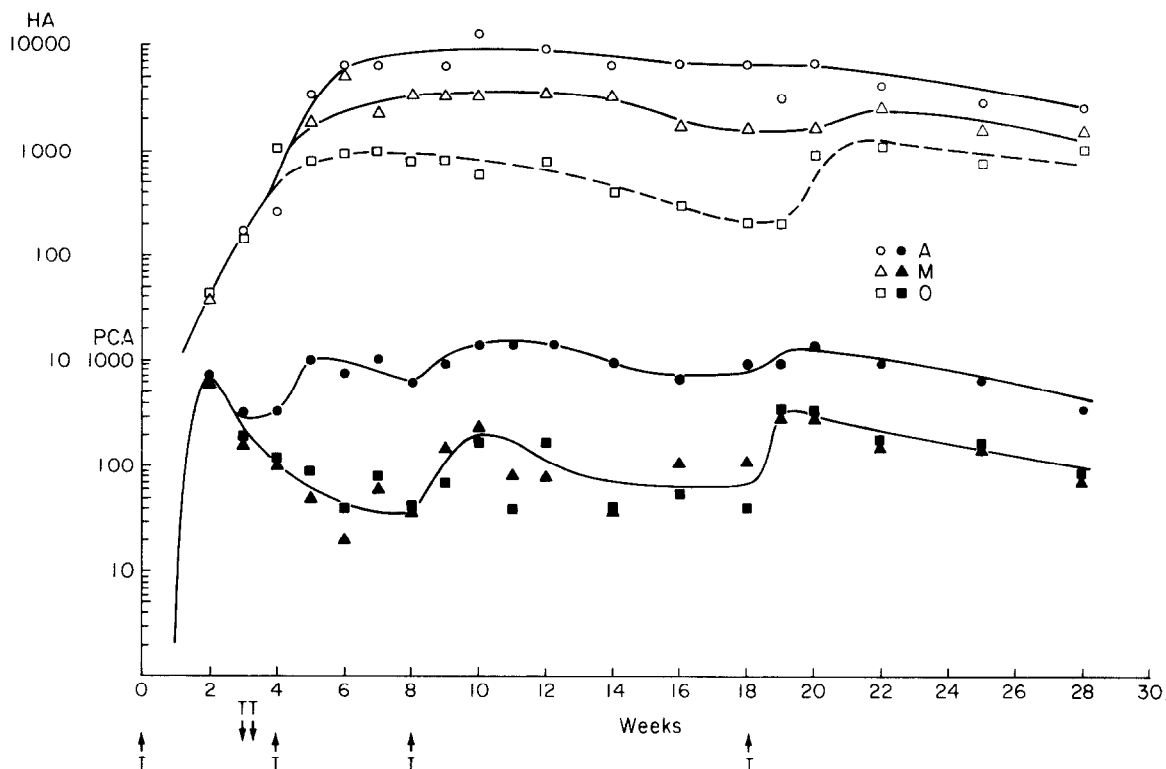


FIG. 1. Immunosuppression of mice sensitized with AgE on treatment with AgE-MPEG conjugate. Mice were sensitized intraperitoneally on day 0 with AgE ($1 \mu\text{g}$, 0.026 nmole) and alum, and treated subcutaneously on days 21 and 24 with the conjugate, a total of 3.6 and 36 nmole for groups M and O, respectively. Group A is the untreated control group. All groups were rechallenged with AgE on weeks 4, 8, and 18. HA (hemagglutination, open symbols) and PCA (passive cutaneous anaphylaxis, filled symbols) denote titers of antigen-specific IgG and IgE antibodies, respectively. Data are taken from reference 30.

We know from studies of other globular protein antigens or synthetic polypeptide antigens that the antigenic determinants specific for IgG antibodies are of the size of 4 to 8 amino acid residues and they are invariably located on the surface of the molecule.²³⁻²⁵ These amino acid residues of an antigenic determinant can be sequentially adjacent to each other in the primary structure of the molecule, or they can be sequentially distant but spatially adjacent in the secondary structure. It is the folding of a polypeptide chain into a compact globular-shaped protein which brings some sequentially distant amino acid residues into spatial proximity. Therefore, the determinants which are composed of spatially adjacent amino acid residues will depend strongly on the native conformation of the molecule.

Although the foregoing data apply to antigenic determinants which react with specific IgG antibodies, it is most likely that they are applicable also to those which react with IgE antibodies. There is little reason to believe that the determinants which elicit IgG and IgE antibody responses are necessarily different. In fact, there is evidence to show that antibodies of IgE,

IgG, and IgM classes can recognize the same antigenic determinant, e.g., in sensitivity to penicillin²⁶ and on immunization with tetanus toxoid.²⁷

As already mentioned, the major antigenic determinants of most proteins are dependent on the conformation of the native molecule. This, apparently, is not the case for the immunogenic determinants which react with T cells. This conclusion is indicated by findings that the denatured antigens or their derivatives, depending on the dose used, could either prime or suppress antibody responses to the native antigen in mice. This is believed to result from activation, by the denatured antigen, of T-helper or suppressor cells. Such studies have been carried out with ragweed AgE²⁸ and ovalbumin.²⁹ These denatured antigens are possible reagents for immunotherapy in man, since they have low allergenic activity and suppress antigen-specific antibody responses.

Another approach to obtaining modified antigens with similar immunologic properties is to attach nonimmunogenic polymers to the antigenic molecule. Steric hindrance of the polymer chains reduces the accessibility of antigenic determinants, thereby de-

creasing the antigenic valency of the protein. Antigenic valency is known to be important for allergenicity and immunogenicity of the protein. Allergenicity depends on the interaction of a multivalent antigen with cell-bound IgE antibodies for release of chemical mediators of immediate hypersensitivity.³⁰ Immunogenicity depends on the interaction of a multivalent antigen with T and B cells^{24, 30} and possibly with macrophages for antibody synthesis.³¹

An example of the above approach is the preparation of conjugates of ragweed AgE with methoxy-polyethylene glycol (MPEG), a random copolymer of D-glutamic acid and D-lysine (DGL), or with mouse IgG. The samples of MPEG and DGL studied possess average molecular weights of 5,000 and 34,000, respectively, as compared with the values of 38,000 and 160,000 for AgE and IgG. MPEG was activated with sym-trichloro-triazine, then coupled with AgE.^{32, 33} DGL³³ and mouse IgG³⁴ were coupled to AgE by intermolecular disulfide bond formation following appropriate activation.³⁵

The conjugates presently prepared have the following probable compositions: AgE(MPEG)₄₋₈, (AgE-DGL)₁₋₂, and (AgE-IgG)₁₋₂. All these conjugates showed reduced allergenic activity in comparison with the native antigen. The immunogenic and immunosuppressive activities of the conjugates were tested in the mouse as the experimental animal. The mouse system is generally accepted as a good model for reaginic antibody formation in man.³⁶ Only results obtained with AgE-MPEG conjugate are described here.

The AgE-MPEG conjugate was apparently nonimmunogenic for mice, but it retained the immunosuppressive property of the native antigen. AgE-sensitized mice treated subcutaneously with AgE-MPEG conjugate had decreased specific IgE and IgG antibody levels about five- to twenty-fold (Fig. 1). The level of depression was dependent on the treatment dose and was long lasting, but was weakened by repeated sensitizations. Comparative treatment data suggest that AgE-MPEG conjugate is a more effective immunosuppressive agent than AgE. Similar findings in mice have also been reported by another laboratory for conjugates of ragweed antigens with polyethylene glycol.³⁷ These results have prompted us to investigate the use of AgE-MPEG in ragweed-sensitive patients for suppression of their IgE antibody levels, studies which are now in progress.

The findings which I have summarized in this presentation indicate that atopic allergens have properties indistinguishable from those of any other protein antigens. One generalization which can be made about the major allergens from different sources is that they

are usually proteins of 20,000 to 40,000 daltons and are usually the chief protein components of the allergen source of interest. This generalization is probably related to the dependence of IgE antibody response on dose, molecular complexity, and retention of the antigen by the susceptible host.

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