

Immunochemical Studies of Ragweed Pollen Allergens

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Hay fever caused by ragweed pollen in late summer to early fall is a very common problem for many people living in parts of the United States and Canada. Ragweed pollen extract contains at least 50 antigens which are detectable on crossed immunoelectrophoresis (8).

By the combined use of salt precipitation, gel filtration and cellulose in exchange chromatography, at least six of these antigens have been purified to near homogeneity (Table 1). Antigens E and K are acidic proteins of about 38,000 daltons (6). The other purified ragweed antigens Ra 3 (9), Ra 4 (5), Ra 5 (3) and cytochrome C (4) are all basic proteins with molecular weights varying from 5,000 to 28,000 daltons. Only antigen Ra 3 has been clearly shown to be a glycoprotein. These six proteins together represent about 11% of pollen proteins, with antigens E and K as the most abundant ones. With the exception of cytochrome C, the biochemical function of these proteins is not known.

Comparative radioallergosorbent test data using sera from 65 ragweed sensitive individuals (1) have confirmed the early observations that antigens E and K are the major allergens since the majority of persons tested showed high serum levels of IgE antibodies specific for these two antigens. Antigens Ra 3, 4 and 5 are minor allergens since the majority of persons tested showed low serum levels of IgE antibodies specific for these three antigens. However, the data do show clearly

that some selected individuals can have high IgE antibody levels specific for these minor allergens which are just as high as those specific for major allergens.

At present we do not know the chemical nature of antigenic determinants of any of these ragweed antigens which react with specific IgG or IgE antibodies. We only know that the determinants of these proteins are strongly dependent on the conformation of the native proteins.

Antigen E was modified by coupling with non-immunogenic polymers, methoxypolyethylene glycol of 5,000 daltons, a copolymer of D-glutamic acid and D-lysine of 34,000 daltons, and immunoglobulin G (2, 7). These conjugates showed greatly reduced allergenic activity, which was measured by heterologous passive cutaneous anaphylaxis in rats sensitized with murine anti-antigen E reaginic sera. The conjugate with methoxypolyethylene glycol was apparently non-immunogenic in mice which are high responders to the native antigen. All conjugates retained the immunosuppressive property of the native antigen since subcutaneous treatment of antigen E sensitized mice with these conjugates led to significant long lasting depression of their antigen E-specific IgE and IgG antibody levels. These immunological changes are believed to result from a reduction of antigenic valency and specificity upon coupling the bulky polymers to antigen E.

Table 1

Physicochemical properties of some known allergens of ragweed (*Ambrosia elatior*) pollen^a

Antigen	MW	Isoelectric point	Nitrogen content	Carbohydrate content	Percent proteins	Ref.
Antigen						
E	37,800	pH 5.0	17.1%	< 0.5%	6	6
K	38,000	5.9	16.6%	< 0.5%	3	6
Ra 3	12,100	8.6		8.0%	0.4	9
Ra 4	28,000	8			0.9	5
Ra 5	5,000	9.5		0	0.1	5
Cytochrome C	13,000	> 10			0.1	4

^a 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 Ra4 is also known as antigen BPA-R.

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Timothy Pollen Allergens

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Among grasses timothy pollen allergens are the best studied. Through a series of studies Augustin et al. (1-4) characterized timothy pollen antigens and demonstrated the existence of allergens with molecular weight about 14,000 dalton and α to β mobilities. Malley et al. (12, 13) later partially purified one of these, denoted Antigen B.

An intensive study of timothy pollen allergens was initiated by Løwenstein, Weeke and co-workers (6, 7, 10, 11, 14, 16) after very powerful tools for the characterization of allergenic extracts — particularly QIE (8) and RAST-technique — but also the more sophisticated separation techniques were made available. During these studies timothy pollen extracts were revealed to be composed of at least 30 antigenic components of which 12 were shown by means of CRIE to react with patients' specific IgE (i.e. act as allergens). Four of these allergens were isolated, partially characterized by physical-chemical methods and their allergenic activities verified by direct *in vitro* and *in vivo* tests on patients.

During the past few years we have continued our studies on timothy pollen extract and have included it in a model for allergen standardization (9); it has also been studied in connection with a clinical comparison between two aluminium hydroxide adsorbed timothy pollen extracts: Alutard[®] SQ and the mixture of two

purified allergens designated antigens Nos. 19 and 25 (15).

It is the aim of this work to repeat the main results of our previous isolation studies and to relate this with our current studies — paying special attention to the identification of allergens by means of CRIE, and the importance of the isolated allergens in relation to clinical experiences.

Four allergens, antigen Nos. 8, 19, 25 and 30 (ag 19 and 25 always remained together) were isolated from freeze-dried aqueous extract of timothy pollen by various combinations of anionic and cationic exchange, and gel chromatography. The allergens were all of protein nature and contained less than 2% (w/w) immunochemically detectable impurities. Molecular weights and pI's were determined to $< 10 \times 10^5$, 15×10^5 , 15×10^5 , 34×10^5 and 3.9, 4.9, 4.5, 9.4, respectively. Antigen Nos. 19 and 25 were determined to be the major components of antigen B.

The allergenic activities were verified by means of RAST inhibition and prick-tests and proved antigen No. 25 to be quantitatively the most important allergen of timothy pollen. Immunologically, this allergen was shown to be partially identical to the most important pollen allergen from three other grasses (7).

The main advantages of this purification study

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