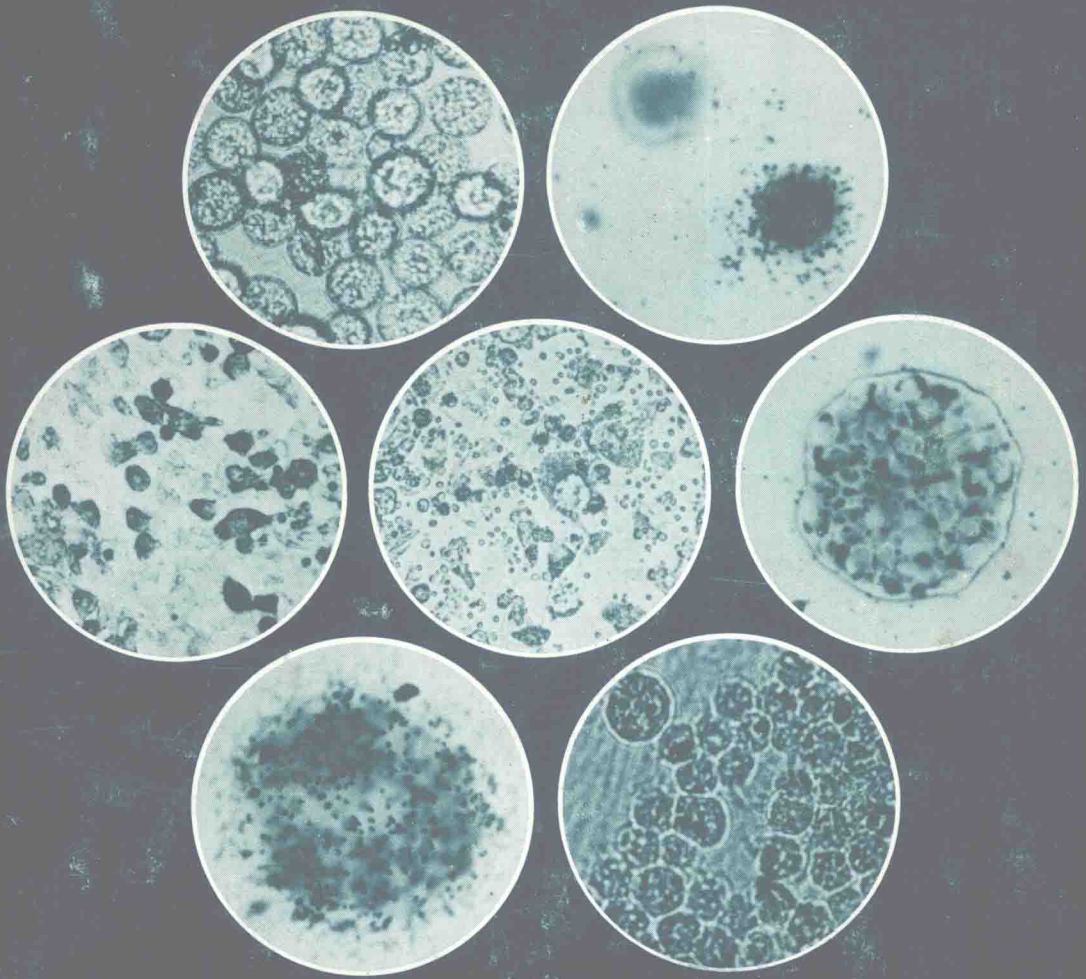
Edited by S.G.O. Johansson, Kjell Strandberg, and Börje Uvnäs

Molecular and Biological Aspects of the Acute Allergic Reaction



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MOLECULAR AND BIOLOGICAL ASPECTS OF THE ACUTE ALLERGIC REACTION

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Introduction

Ladies and Gentlemen, dear guests,

It is my great pleasure and privilege to extend our heartiest welcome to you, the participants of this 33rd Nobel symposium. To those of you who have not attended a Nobel symposium before I would like just briefly to explain why Nobel's name is linked to this series of symposia. Alfred Nobel, who died in San Remo in 1896, donated the main part of his fortune to the promotion of international science and culture by establishing annual prizes for outstanding discoveries or contributions within five fields, chemistry, physics, physiology or medicine, literature and peace. The annual awards should be distributed by five corresponding prize committees out of which four in Stockholm and one in Oslo (at that time Sweden and Norway were a united kingdom). The Nobel Foundation was instituted in 1900 with the main function to administer economically the Nobel Donation. It has done so very successfully. The Foundation has even been able to beat the inflation and the prizes have steadily increased during the last 20 years. It might interest you to hear that this year's prizes will amount to 681.000 Sw. crowns each.

Due to the favourable financial development and also due to additional donations the Nobel Foundation decided to extend its interests by promoting the arrangements of symposia, lectures and other international scientific activities. Our symposium of today is one of the results of these increased activities and the Nobel Foundation is the main sponsor of our meeting. Some contribution has also been given by the Nobel Committee for Physiology or Medicine which as you might know is elected by the Medical Faculty of the Karolinska institute. The topics chosen for symposia within the medical field must have the approval of this Nobel Committee.

The title of the today's symposium reflects the intention of the organizing committee to arrange a meeting on a topic within the front line of research in experimental allergology. Immunochemistry is since some years a field of exceptionally rapid development.

Advances within this field will no doubt lead to fundamental discoveries for our understanding and therapy of various diseases due to disturbances in our immunological mechanisms, including those on an allergological basis. Even if the main emphasis has been laid on various aspects of immunochemistry, also the target cells for allergic reactions have received their share. It is the hope of the organizing committee that the communications and discussions during the coming three days will be profitable and enjoyable to all of us and will enable us to return home with new knowledge and enthusiam for additional years of successful research in experimental allergology at all levels and to allow us to meet again within a few years for a new fruitful time together in the sign of our patroness Minerva.

Ladies and Gentlemen, I am quite sure that Alfred Nobel should have been very pleased to be present at a symposium of the kind we have ventured to arrange. However, as it is you have to be content with hearty welcome from a humble of the Nobel machinery.

Börje Uvnäs
Chairman of the organizing committee

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Structure of Allergens and Regulation of IgE Immune Response

COMMON CHARACTERISTICS OF MAJOR ALLERGENS

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This discussion will be restricted to the naturally occurring allergens that elicit immediate hypersensitivity reactions when combining with homologous IgE antibodies in human individuals. It is not my intention to review the literature. Comprehensive reviews may be found elsewhere (1,2,3). I will rather focus attention on information thought to be particularly important as basis for future research. Allergen chemistry will only be described in detail when necessary for elucidation or illustration of particular points of interest.

The field of allergen chemistry is a difficult one. One depends ultimately on the biological tests related to the clinical allergy in question. This introduces many methodological problems. Due to the extreme sensitivity of the biological systems in question (4), otherwise undetectable contaminants such as undefined allergenic molecules, irritants or both (5), may be responsible for the reactions recorded. Furthermore, due to manipulations necessary for the biological tests, the material may undergo important changes with effect on the allergenic activity (aggregation, polymerization, conformational re-organization).

All scientific data up to the present time show that naturaly occurring allergens are proteins. However, only a limited number of numerous proteins found in allergenic material and in allergenic extracts are important for the allergic reaction. Only one

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or a very few of the allergenic proteins present in a given extract act as denominator of the allergic reaction in the majority of patients allergic to the matter. They are called "major allergens". In addition, the same extract may contain other allergenic proteins called "minor allergens", which are important only in a small number of the patients. Most patients reacting to any of the minor allergens usually react also to the major allergens present in the material, but for occasional patients the minor allergen may be the most active one.

The question naturally arises why certain proteins such as, for instance, antigen E in ragweed pollen (6) and allergen M in codfish (7) act as major allergens while several other proteins in the same material do not. Much laboratory work has been devoted to efforts to point out characteristic traits that make allergens allergens. Progress is steadily being made in isolation and characterization of allergenic proteins. New methods in protein chemistry have been the most important catalysts in this process together with refined immunological techniques.

Investigations in allergen chemistry must start with ultrapurified and well-defined material. They must make use of methods with extreme capacities of discrimination and with an exceptionally high degree of sensitivity. Extreme care must be taken for the control of the specificity of biological reactions (8,9). With this in mind, the number of allergens and allergen studies rendering substance for this discussion is brought down to a very few. Information derived from studies of, for instance, house dust allergens is too confusing to be brought into a discussion meant to be clarifying (10).

Molecular size and bridging of IgE antibodies

There is some evidence that the initiation of the allergic reaction resulting in mast cell histamine release depends on the bridging of two IgE antibody molecules by the allergenic molecule in question (2,11,12). Many authors have discussed the importance of molecular size for this bridging, and several statements have been made that a molecular size of 10.000 - 70.000 or more restricted between 10.000 and 40.000 is a common characteristic for major atopic allergens (1,2,3). This is substantiated by the actual analyses of a few purified or highly purified allergens (table I). However, if such bridging is critical, it must depend on the number and distribution of allergen determinants accessible on the surface of the molecule and not on the molecular size as such.

Table 1. Range of molecular weights (m.w.) for allergenic molecules and biologically active allergen fragments.

ALLERGEN	m.w.	ref.nr.
Ragweed pollen, E	37.800	(6)
Horse dander	34.000	(13)
Cat epithelium	32.000	(14)
Cat epithelium	55.000	(15)
Rye grass pollen, BI	27.000	(24)
Honey bee venom*	19.500	(16)
Ascaris suum	18.000	(17)
Ascaris	14.000	(18)
Allergen M, cod	12.200	(26,50)
Allergen TM I	8.490	(19,26)
Allergen TM II	3.850	(26,52)
Allergen N (4.85)	3.460	(19)
Ragweed Ra 5	5.100	(20)

* Phospholipase A

The demonstration of prominent allergenic activity of the small fragments TM I (mw 8.492) and TM II (mw 3.854) from codfish (19) as well as the demonstration of a high allergenic activity in some individuals to the dialyzable polypeptide Ra 5 (mw 5.100) isolated from ragweed pollen (20) indicate that quite low limits must be taken into account in statements on which molecular sizes are necessary to induce allergenicity. The upper molecular weight limit of 40.000 or 70.000 may be due to limiting factors in mucous membrane permeability in the allergic host.

Stanworth (2) suggests that Ra 5 and allergen M may represent monomeric subunits which have become dissociated from a dimeric allergen during their isolation. He suggests that bridging occurs when these subunits reassociate under physiological conditions. There is, however, no evidence in support of this suggestion.

Molecular charge

The net charge of a polypeptide or protein depends on the ratio of acidic (Glu, Asp) to basic (Lys, Arg, Hist) amino acid residues, and can be estimated when the amino acid composition is known. Isoelectro-focusing and other methods for the separation of proteins according to the molecular isoelectric points (pi) introduced means to define the net charge of the allergenic molecules more precisely (table 2).

Table 2. Differences in net charge (iso-electric points) between various allergens.

Allergen	isoelectric point
Horse dander	4.1
Codfish, M	4.75
Ascaris suum	4.8 -5.0
Ragweed pollen, E	5.0 -5.1
Rye grass pollen, BI	5.15-5.25
Ragweed, Ra 5	9.6
Phospholipase A	10.5
Detergent alkalase	basic

Most major allergens characterized with respect to pi are distinctly anodic ones with a pi range from 2-5.5. The codfish allergen M has its pi at 4.75, and pi of most pollen allergens is found around 5. The house dust allergen reported by Berrens is claimed to have its pi at 3.1 (1). Notable exceptions are the basic proteins from the detergent enzyme allergen alkalase with a pi around 9, and the basic allergens of cotton seed and castor beans (22), as well as the minor allergens Ra 3 with pi 8.5 and Ra 5 with pi 9.6 from ragweed pollen (20.23).

However, one may assume that it is not the net charge but the charges of the limited combining sites of the molecules that are significant for the binding to the complementary combining site of the IgE antibody.

Iso-allergens

The term "iso-allergens" was introduced by Johnson and Marsh (24) for two major allergens (alpha and beta) found in rye grass pollen. The two allergens were homogeneous and immunologically identical. They were also almost identical in molecular weight, amino acid composition, carbohydrate composition and peptide fingerprints, but differed slightly in their amide content. Similar observations were made by Tangen and Nilsson in studies of timothy pollen allergens (25). Identical allergenic activity was found in codfish proteins with distinct isoelectric-focusing mobilities in acrylamide gels (26). They were considered to be iso-allergens, being proteins with identical allergenic activities, but differing in amino acid composition and net charges with pi 4.75 and 4.85, respectively.

Antigen E appears to be present in ragweed pollen extract in four chemical forms. Two major forms designated as B and C are immunologically identical and have identical amino acid compositions but differ slightly in isoelectric points (27). Similarly, allergenic activity was found in several fractions of house dust mites separated by liquid isoelectric focusing. A high degree of cross-reactivity was found between fractions having very dissimilar pi, varying from 3.0 to 6.4 (28).

The results of the latter studies strongly suggest that the extract contains one or only a few main allergens existing in multiple molecular forms rather than several distinct allergens. Multiple molecular forms may arise during extraction and during fractionation manipulations with changes of the immediate environment of the molecules, or they may exist in the original form of the proteins. Effects of this kind may be important also for the occurrence of IgE-binding antigens with distinct mobilities which can be demonstrated in cross-radio immunoelectrophoresis of allergenic material (29). Following investigations making use of such techniques, Weeke and co-workers (29) stated that timothy pollen extracts contain at least 11 distinct allergens. It remains to be shown whether some of them are iso-allergens or not.

Stability and structure

Many allergens are remarkably stable to chemical denaturation, but this is not a common trait and marked differences are found in susceptibility to different agents (table 3). Pepsin digestion does not impair the activity of rye grass (Lolium perenne) pollen (3), but readily destroys the allergenic activity of antigen E from short ragweed pollen (30). The codfish allergen is also inactivated by hydrolysis with pepsin as well as with trypsin, but the time needed for complete inactivation was found to be 2-3 hours in standard experiments (7). Hydrolysis for shorter periods resulted in products still active. Reactivity decreased with length of the hydrolysis. Allergenicity and antigenicity in immunodiffusion with homologous rabbit antisera disappeared simultaneously.

Antigen E is readily inactivated also by other denaturing processes such as heat, whereas the rye pollen major allergen, group I, resists boiling for 30 minutes, changes in pH and treatment with 8M urea (3). The major allergens in birch pollen also resist heating for a prolonged time (31). In contrast to antigen E, the codfish allergen is remarkably stable to prolonged heating, to pH changes and to denaturing agents such as 8M urea. The codfish allergen differs also from other allergens as regards susceptibility to physiochemical degradation. Whereas group I allergen from rye grass pollen loses most of its allergenic activity following mild formalin treatment (32), the allergenic activity of codfish was retained unaltered or only slightly reduced following

Table 3. Differences in allergen resistance to denaturing agents as illustrated by three allergens.

IMMUNOCHEMICAL CHARACTERISTICS OF MAJOR ALLERGENS

	Allergen M (cod)	Antigen E (ragweed pollen)	Allergen IB (rye pollen)
Stability to			
heat	+	-	+
freeze-drying	+	(+)	+
freeze and storing at -20°C	+	-	+
acids	+	-	+
tryptic digestion	(+)	(+)	(+)
peptic digestion	(+)	-	(+)
formalin treatment	+	-	-