

What Makes an Allergen an Allergen

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In the vocabulary of daily life the term allergen is most often used to indicate the whole substance able to initiate an allergic reaction, such as certain pollens, moulds, house dust mites, animal dander, cow's milk and other inhalants or food items known to trigger immediate or late hypersensitivity reactions. The term is also used to denote environmental components which elicit delayed hypersensitivity reactions. Allergen also denotes the particular molecules which specifically take part in the immune reactions of allergy.

The present discussion will be restricted to the naturally occurring allergens that elicit immediate hypersensitivity reactions when combining with homologous IgE antibodies in human individuals. In the following, the term "allergy", "allergic" and "allergen" refer to this restricted field. Data from a large number of investigations have been utilized in this presentation, but reference is made only to a few selected papers summarizing the most important information in this field (see 3, 5, 11, 13, 14).

Why do some substances and some molecules act as allergens while others apparently do not? Answering this question may provide information about some basic mechanisms of allergy and about molecular and cellular aspects of allergic diseases, and at the same time aid the development of new

and better diagnostic methods in the laboratory. It may lead to more effective therapeutic approaches. All this should give a strong inducement to research work in this area.

The immunological reaction

The definition as well as the demonstration of the existence of an allergen depends on an immune reaction occurring in a living organism. The immediate hypersensitivity reaction in human individuals is initiated by the interaction of two equally important components, the allergenic molecule and the corresponding antibody molecules of the immunoglobulin E (IgE) class. Before this class of immunoglobulin was defined (9, 10) the type of antibody was known as reaginic antibody, reagin, skin sensitizing antibody or anaphylactic antibody.

One prominent characteristic of IgE is its property to bind to specific IgE receptors on mast cells and basophil granulocytes of the same or closely related species. Antibodies of this type are called homocytotropic antibodies. Each basophil cell may carry from a few thousand up to one million receptors for IgE on its membrane.

The combination of cell-bound IgE antibodies with the complementary allergenic molecules is essential for the allergic reaction, whereas allergen combining with circulating antibody is at the present not known to have any biological significance. The immune reaction on the mast cell

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surface triggers certain intracellular events, resulting in the release of biochemical mediators and agents which are able to activate extra-cellular mediator systems. The mediators act as excitatory stimuli on smooth muscles and glands, resulting in quite complex allergic inflammatory tissue reactions (1, 4). The mediator release may to a certain extent be regulated by complex biochemical feed-back mechanisms and by a number of drugs.

Incidence of allergy

Low concentrations of IgE are normally present in all individuals both on mast cells and in serum, and all of us are exposed to a vast number and variety of environmental antigens. It is, however, only a proportion (10–30%) of the population which becomes allergic with overt clinical symptoms under natural conditions of exposure (allergic, or “atopic” individuals). It is conceivable that the rest of the population (the so-called non-allergic or “non-atopic” individuals) also respond with IgE antibody synthesis in some way or other to a number of stimuli, but without recognizable disease. IgE antibodies without clinical significance are found also in allergic individuals, particularly to a number of food components, and can be raised in non-allergic individuals following repeated infections with allergenic material. IgE without demonstrable antibody activity is sometimes referred to as “physiological IgE”, a term which could equally denote IgE antibodies not responsible for clinical disease.

Otherwise non-allergic individuals have been shown to produce IgE antibodies and exhibit allergic reactions following extreme exposure. Since daily life can entail different degrees of exposure to a variety of agents from the normal to the extreme, this may partly account for the broad range of incidence reported for hypersensitivity reactions and allergic diseases. In general, major allergic diseases are said to be found in 5–10% of many populations, and minor

allergic complaints in an additional 15–25%. Differences in reported incidence may also result from differences in the definition of clinical entities and lack of standardization of diagnostic criteria, material and methods.

Host factors

Only a minor although substantial proportion of human individuals exposed to the same environmental substances develops allergies. This indicates that host factors contribute to or determine whether a given environmental substance or certain molecules act as allergens or not. Important host factors in this respect appear to be the immune responsiveness (IR) within different systems, and particularly within those involving IgE. In addition, a number of non-immunological, or so-called “conditioning” factors appear to influence the localization and degree of immunological sensitization, as well as the type and degree of clinical manifestation (2, 12).

The responsiveness in the IgE system is thought to be influenced by at least two distinct genetic factors, i.e. by genes regulating the general responsiveness in IgE production as such, and by genes regulating the antigen specific response. Those trying to elucidate the genetics in this field have to face a prominent complexity of interactions between different immunoglobulin systems and the collaboration or competition between different immunocompetent cells during varying stages of stimulation (13, 15).

Induction of antibody formation to a variety of antigens (immunogens) requires the collaboration of thymus-derived (T-cells) and bone-marrow derived (or Bursa-Fabrics equivalent) (B-cells) lymphocytes. Following trigger signals from the particular antigen, so-called T Helper cells stimulate the proliferation of B-cells into antibody-secreting plasma cells (“on”-signal). T Suppressor cells inhibit or block this function (“off”-signal). The interaction between T Helper, T Suppressor and B-cells is regulated by a number of unknown factors. However, a

series of experiments indicate that in some animals low-dose stimulation favours persistent IgE production, whereas high-dose stimulation favours IgG, and in other animals the reverse is true. The type of response is genetically determined (9, 13, 15).

What is inherited is the coded information for the composition and structure of a polypeptide, and the polypeptide participates under certain circumstances in forming a given trait. B-cell structure genes determine the type of immunoglobulin and the specificity of the antibody to be produced. The polypeptide in the cell membrane reacts with a defined complementary structure which fits stereochemically with it, and functions as the antigen for that cell. The particular antigen signal triggers the cell to proliferation and antibody production. This immune responsiveness (IR) to various antigens is controlled by IR-genes which may or may not be linked with HLA-associated genes. IR-genes give the coded information for a polypeptide in the T-cell surface; that is, IR-genes determine antigen recognition at T-cell level with a high degree of specificity. T-cell and B-cell collaboration is necessary for a specific antibody to be formed. Regulatory T-cells appear to play a fundamental role in the synthesis of IgE antibody (15).

Only those whose genetic code is strongly programmed to produce IgE antibodies will do so under the conditions of normal low-dose environmental inhalant or food exposure. If not so programmed, the individual produces IgG antibodies that serve to neutralize and bind the antigens in question, which are then cleared by the reticulo-endothelial system. However, also non-immunological factors seem to be necessary for the development of allergic disease, namely a biochemical defect of some sort resulting in overreactivity of the tissues in question (1, 2). Interesting recent data indicate important influences of biochemical mediators, released in allergic reactions, on the function of regulatory T-cells, as well as interactions between biochemical systems

responsible for receptor reactivity, both in the allergic tissues and in T- and B-lymphocytes.

A number of environmental antigens, in addition to the so-called naturally occurring allergens, may induce IgE antibody production and allergic reactions under extreme environmental exposure or the appropriate experimental conditions. The fact that a number of naturally occurring antigens, although present in high concentrations in the environment, do not induce allergies under normal conditions, indicates that there may exist certain host factors which serve to exclude them. However, protective host factors may be by-passed under experimental conditions or broken down during disease, giving room for more rare allergies to occur.

In general, many efforts have been made to explain the host factors responsible for the development of atopic allergy in man (12). Also many non-immunological tissue factors have been implicated as host factors important in determining whether a substance or molecule shall act as an allergen or not. It appears that even genetically linked host factors may be modified to a certain extent by environmental factors or by active interference. The degree and type of stimulation by antigen are factors which influence which function may eventually be put into action and which trait will be the dominant one. A number of data show that the activity of immunocompetent cells may be influenced and regulated by a number of mediators of inflammation, allergy and neurophysiology, as well as by drugs. This can be interpreted as evidence that the interaction of inherited and environmental factors are of importance for determining what makes an allergen an allergen in a given individual.

Environmental influences

The early studies on inheritance of allergy indicated that sensitized individuals do not transmit to their offspring their own sensitization regarding antigen specificity or particular tissue involvement. More recent

investigations tend to show that type of disease, and sensitization to a particular substance is inheritable, but by and large confirm the original statement that it is mainly the disposition to become allergic which is inherited (2, 13). Most of the IgE responders (and most non-atopic individuals as well) probably have the ability to respond to the same immunogens found in common environmental substances.

Infestation with intestinal parasites gives rise to extremely high serum concentrations of IgE and specific IgE antibodies, both in man and animals. Most human individuals with past or present helminthic infestation react with reaginic (IgE) antibody production and positive skin test wealing to extracts of the parasite, irrespective of whether they are otherwise allergic or non-allergic. Ascaris and ascaris allergens are particularly well known and have been carefully studied in this respect. It has been speculated that this type of allergen represents the evolutionary origin of its kind, making IgE play the physiological role in the expulsion of intestinal worms in animals and the ancient cave-dwellers.

Also non-immunological factors in the environment may have some influence through adjuvant effects, or exert changes in tissue permeability (smoke and other irritants) or host defence mechanisms (infection). Tissue permeability to macromolecules may be a crucial factor in this respect. Changes in tissue permeability favouring sensitization may result from microbiological or allergic inflammation, from chemical or physical irritation, and neurophysiological stimulation. The individual or tissue with increased vascular permeability for the antigens in question may be more readily sensitized to even low exposure of the immunogenic agent.

Allergen exposure

The moulds *Cladosporium herbarum* and *Penicillium notatum* are characterized by their omnipresence, and allergy to these moulds

are found almost everywhere in the world. Allergy to ragweed pollen, however, is found exclusively in individuals who are exposed to this kind of pollen in restricted regions in the United States, but not among inhabitants of, for instance, Scandinavia. On the other hand, the birch tree is widespread in Scandinavian countries and birch pollen allergy is quite frequent in Scandinavian individuals, but rare among inhabitants of New York. Allergy to dog epithelium is frequently found in populations keeping dogs as household pets, but allergy to rat hair is found mostly among laboratory staff who handle this rodent. Norwegians are big fish-eaters, and codfish is prepared in many places in the fish industry. Consequently, fish allergy is frequent in Norway, but does not occur among the beef-eating inhabitants of Texas. Innumerable other examples can be given of the necessity of exposure for an allergen to become an allergen. The substance must be present in substantial amounts, and preferably over prolonged periods, in the patients' environment or food.

On the other hand, there are many substances which are quite abundant in inhaled air or in food, but do not cause allergy. Pine pollen is an example of this in the Scandinavian countries. The reason they do not cause allergies is explained by the structure of the pollen grain. The pulp of the antigenic proteins are encased in a tough cellulose layer which is resistant to the enzymes of the human respiratory passages. The pollens are excluded from the respiratory passages and no antigenic material comes in contact with the mucosa.

Innumerable quantities of bacteria and viruses are normal or occasional inhabitants on the skin and mucosa. The possible role of viral and bacterial components as allergens is controversial. Although it is not unlikely that bacterial and viral components, or infectious products, may act as natural allergens, there is no convincing proof that they do this under natural conditions. It has, however, been shown that bacterial components and

products may give rise to immediate hypersensitivity and specific IgE responses under extreme conditions. Enzymes from *Bacillus subtilis* which are industrially produced for large-scale use in detergents, are one example of this. Dextran, which is a polymer of D-glucose produced by certain bacteria, has been found to cause generalized allergic reactions of the immediate type caused by reagin-like antibodies in some people. These are, however, examples of bacterial products used in extremely high concentrations and volumes, and cannot be used as evidence in the debate. Bacterial and viral infections are not followed by increase of total serum IgE levels as is found following exposure to known allergens, and most or all of the exacerbations of allergic symptoms caused by infections in susceptible individuals can be explained by non-allergic mechanisms. However, the final answer has not yet been given and the question calls for meticulous and critical research with the use of modern methodology. Non-pathogenic saprophytes are as likely candidates as the pathogens in this respect. If they do not act as allergens, this can certainly not be due to lack of mucosal exposure.

The ease with which the antigenic material reaches the mucosa seems to be one of the factors that determine what makes an allergen an allergen. For pollen, the rate of release from the pollen grain may have an important bearing on the allergenicity of different proteins found in the grain. The major allergen of birch tree pollen and the most important rye grass pollen allergens are shown to be released very rapidly from the pollen grains.

However, the protein considered the most important allergen in ragweed pollen, Antigen E, is released more slowly from the pollen grains than other proteins of less importance. This may be interpreted in different ways. One interpretation is that Antigen E has molecular features with much higher allergenic potential than the others. Similarly, differences in the allergenic potential of different proteins may account

for the fact that allergy to fish is more common than allergy to meat, even in families eating more meat than fish. These and other data suggest that it is not only the mode and degree of exposure but additional molecular characteristics which make an allergen an allergen.

Molecular characteristics

Usually, a limited number of the numerous components found in allergenic material and allergenic extracts is important for the allergic reaction. Whereas antigenic activity in IgA, IgM and IgG may be associated both with proteins, carbohydrates and – to a limited extent – lipids, allergenicity in IgE is exclusively associated with proteins or polypeptides, as far as we know at the present time.

Only one or a very few of the allergenic proteins or polypeptides present in a given extract act as denominators of the allergic reaction in all or most 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 for only a small number of patients or of little importance for all of them. Most patients reacting to any of the minor allergens usually react also to the major allergens present in the material, but for some patients the minor allergen may be the most active one. The presence in different substances of similar minor (or rarely, also major) allergens accounts for varying degrees of cross-reactivity, e.g. as seen between grass pollen and wheat flour.

The question naturally rises as to why certain distinct proteins, such as for instance "Antigen E" in ragweed pollen and "Allergen M" in codfish, act as major allergens while several other proteins in the same material do not.

Much laboratory work has been devoted in efforts to point out characteristic traits that make the protein molecules allergenic (3, 5, 11). 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 for discrimination and an exceptionally high degree of sensitivity. Extreme care must be taken in the control of the specificity of biological reactions. Due to the sensitivity of the biological system in question, otherwise undetectable contaminants such as undefined allergenic molecules, irritants, or both, may be responsible for the biological reactions recorded. Furthermore, due to manipulations necessary in biological testing, the material may undergo important changes with effects on the allergenic activity such as aggregation, polymerization, and conformational re-organization.

All scientific data up to the present time show that naturally occurring allergens are proteins or polypeptides. However, the possible role of carbohydrate antigens, and particularly those of bacterial and viral origin, is far from elucidated. Furthermore, modern industry has provided our environment with an almost indefinite number of agents, some of which appear to elicit immediate type allergic reactions probably involving IgE antibodies as well as other immunological mechanisms. The agents in question probably act as allergens after being bound or conjugated to proteins or peptides of the allergic host. This is an increasing problem in pharmacology, and not least in the industries most troubled by occupational allergy. To solve problems in this field, information is needed about the characteristics of the allergenic molecule and antigenic and allergenic determinants.

Antigenic determinants are defined as the discrete areas of the molecule surface structure that combine specifically with the complementary discrete areas of the particular antibody. Allergenic determinants are defined as the discrete areas of the

protein surface structure that combine with the complementary discrete areas of the particular antibody taking part in the hypersensitivity reaction. In our limited context allergenic determinants are the antigenic determinants which combine with IgE antibodies.

Antigenicity and allergenicity

Most commercial allergen extracts in use are made by very simple procedures. They are complex mixtures containing several proteins, peptides, carbohydrates and other soluble substances. New technical developments in protein chemistry and in the immunological sections of experimental allergy have provided fine tools for further exploration of antigenic and allergenic components and their characteristics. Various modifications of radioallergosorbent tests (RAST) and RAST-inhibition have replaced the passive transfer test of Prausnitz and Küstner. Other techniques such as crossed immunoelectrophoresis (CIE) and crossed radioimmunoelectrophoresis (CRIE) with different modifications enable us to study details of the antigenic material. These techniques make possible a variety of immuno-acrobatics which aid in the identification, characterization and, subsequently, the isolation of allergenic molecules.

Crude extracts from timothy pollen have been shown to contain at least 29 antigenic components, and 11 of them are thought to represent allergens of varying importance. A *Cladosporium herbarum* extract reacts with its homologous rabbit antiserum giving 57 distinct precipitates, only very few of which bind human IgE antibodies. Most of the antigenic proteins present are not allergenic at all in the IgE system of most patients.

This is an *in vitro* illustration of the fact that some components seem to produce sensitization* in the IgE system and give allergic reactions much more readily than others in a way that can not be explained by the degree of exposure alone.

It has been stated that allergens are weak immunogens and antigens owing to their

ability to react with antibodies raised in animals (usually rabbits) immunized with the allergen extract in question. For low molecular weight molecules this would not be surprising since large molecules are known to be better antigens than smaller ones. Nevertheless, a number of allergens have been shown to be quite good antigens, at least in a number of the rabbits immunized. The immunogenicity and antigenicity probably depend partly on the immune responsiveness of the animals used, and on the presence in the different molecules of amino acids which are particularly active as immunodominant components, such as aromatic amino acid residues in combination with polar amino acid residues (8). From available data we can conclude that some allergenic molecules (in the IgE system) are weak antigens (in the IgG system) while others are good antigens. It should be emphasized, however, that the allergenic and antigenic sites (determinants) active in the two systems are not necessarily identical even if they are found on the same molecule. One molecule may even carry different allergenic sites just as it represents a mosaic of different antigenic sites.

Molecular size and bridging of IgE antibodies

There is much 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. 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 often between 10,000 and 40,000, is a common characteristic for major atopic allergens. However, if bridging is critical, it must depend on the number, distribution, and distance between accessible allergenic determinants in relation to the distance between the homologous IgE antibodies on the mast cell surface, and not on molecular size as such.

Many workers in the field seem to accept the implication that the allergenic determinants on the molecule have to be identical for bridging of IgE antibodies to occur. However, no data are available showing that this is a prerequisite. Indeed, there is a hypothetical possibility that non-identical determinants on the molecule may be just as effective and that this may partly explain some cross-reactivity phenomena.

Small polypeptides have been shown to be biologically active allergens in some individuals (3). Provided that aggregation or polymerization following introduction of the peptides into the skin can be excluded, this indicates that low limits must be taken into account in statements on which molecular sizes are necessary for biologically active allergenicity. Otherwise, low molecular weight substances frequently act as monovalent – haptenic – determinants.

An upper molecular weight limit of 40,000 or 70,000 for naturally occurring allergens may be due to limiting factors in mucous membrane permeability in the allergic host. Larger molecules may induce IgE antibody formation following parenteral administration.

Molecular charge

The net charge of a polypeptide or protein depends on the ratio of acidic (Glu, Asp) to basic (Lys, Arg, His) 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 (3, 5, 13). However, one may assume that it is not the net charge, but the charges of and around the limited allergenic determinants that are significant for the binding to the complementary combining site of the IgE antibody.

Differences in the charge of allergenic molecules may give rise to multiple molecular forms. Deamination, slight con-

formational changes, or other factors, may give molecular forms with different mobilities in an electric field, but carry the same allergenic determinants and overall traits necessary for combination with the corresponding IgE antibodies. Multiple molecular forms may (a) exist in the original form of the protein or (b) arise during extraction and during fractionation, and with changes of the immediate environment of the molecules. Some of them may be considered to be iso-allergens as defined by Marsh (13).

Stability and structure

Antigenic components of environmental agents and food substances are subject to a number of denaturing processes before they reach the immunocompetent cells or cell-bound antibodies in the human organism. Antigens resisting the host defence mechanisms are the most likely ones to elicit immune responses, and many allergens are remarkably stable to enzymatic and chemical denaturation. It was known already 70 years ago that the main skin reactivity of rye grass pollen resided in an albumen fraction and was rather stable to extreme acid or alkaline pH and to enzymatic degradation and heating. In fact, Noon who introduced hypersensitization with allergen extracts more than 50 years ago showed that grass pollen extracts were active following sterilization at +100°C for 10 min. However, this is not a common trait. Marked differences are found in susceptibility to different agents. It was known 50 years ago that most of the activity of ragweed pollen extracts is destroyed on boiling the extract. However, many of the other antigens present in the allergenic substance and in the crude allergen extracts are less resistant to denaturation than the allergenic molecules.

Effects of denaturation in different tissues may also determine differences in response to a number of allergens. This is illustrated by allergy to cereals. A large number of bakers become allergic to wheat. However, in spite of having excess concentrations of

IgE-antibodies both in serum and in the skin, they react only (with respiratory symptoms) when inhaling wheat flour dust and do not react when eating wheat. Other allergens, such as green peas and codfish, elicit allergic reactions both when ingested and when emanations or dust from them are inhaled.

Denaturation involves changes in protein structure. Differences in stability may reflect differences in structure. A protein or polypeptide is made up of a number of amino acids bound together in the primary structure. Each amino acid has a side chain which is characteristic for it, and these side chains together represent chemically active sites on the protein molecule. The whole molecule is given its final shape through conformational changes of the polypeptide chain. The chain is twisted and folded up in its secondary and tertiary structure, respectively, due to forces exerted by the different side chains.

It is the side chains of a few amino acids brought together by the folding which most often constitute the antigenic determinants. Antigenic determinants may be so-called "sequential" or "conformational" ones. A sequential determinant is organized from a number of amino acid residues as found directly in a linear sequence in a random coil (unfolded) form of the molecule, and accessible as such on the surface of the molecule in its natural form. Antibodies to this type of determinant are expected to react with a peptide of identical or similar sequence (6, 8). A conformational determinant results from the steric conformation including amino acid residues that are remote in the unfolded polypeptide chain, but are found in juxtapositions in the native structures. Most protein determinants that interact with humoral IgG antibodies are thought to be conformational, but sequence dependent antigenic determinants have been demonstrated for many polypeptide and protein antigens (7).

Only those accessible on the surface of the folded molecule can interact as antigenic or

allergenic determinants with the particular antibody. The number of accessible allergenic determinants per molecule may be the factor which determines the potency of the particular allergen in addition to the binding dynamics.

Certain structural features may be particularly important for the immunogenicity of a molecule at the surface levels of macrophages, T-cells and B-cells, and when reaching cellbound IgE antibodies. Molecules with a high degree of resistance to enzymes and other degradation processes exerted on them by living cells and membranes, with a high structural complexity, and with an optimal number of immunogenic side chains appear to be the best candidates in this respect (7).

We would like to know what allergenic molecules look like from the outside – an antibody's-eye view, so to say. However, detailed descriptions of conformational determinants can only be derived for proteins whose tertiary structure has been resolved by X-ray crystallography at the atomic level. This has not yet been done for isolated allergenic proteins with defined allergenic determinants. However, even when the tertiary structure of a protein is determined, this gives only a picture of the most probable conformation in the crystal. It still remains to know how far this structure represents that of the molecule in solution, and when it reacts *in vivo* with the particular antibody.

Single residue as obligatory feature

A single amino acid residue or a particular prosthetic group may be of crucial importance in defining the activity, even when acting only as part of a larger antigenic determinant, whereas other residues may be important only in the complementary stereological fit between antigen and antibody without directly taking part in the binding. Some information is available for a discussion of the general importance certain single residues have for allergenic activity. By

comparing the available amino acid compositions of a number of allergens, the role of many of them as a single component determinant can be ruled out (3).

It has been suggested that the presence of *N*-glycosidic linkages is a characteristic trait of allergens. The almost universal role of lysine-sugar structures for different molecules to act as allergens has been postulated (5). The lysine-sugar site was thought to be involved in priming the allergic reaction in a non-specific way, as a kind of adjuvant. It was called an "allergenic site" without implying that it should act as an antigenic (or allergenic) determinant in the immunological sense. This postulate has lost some of its substance since the progress in allergen purification and characterization. Naturally occurring proteins usually contain carbohydrate more or less tightly bound to their amino acid side chains, and it has been shown that *N*-glycosidic linkages are insignificant for allergenic activity in a number of allergens, including codfish Allergen M. *N*-glycosidic linkages may play a role in the immunogenicity or allergenic activity of some molecules, but this can not be considered a common characteristic. It appears that there is no proof that any single component, such as a given amino acid or sugar derivative, is essential for an allergen to be an allergen (3, 13).

On the other hand, the importance of, for instance, Ca^{2+} and Mn^{2+} or various metal ions for the binding capacities between allergenic and antibody molecules is not understood or even been investigated. Such ions have a marked influence on both the chromatographic behaviour and the biological activity of a number of proteins, and they may be important for the structural organization of the polypeptide chain. It is unlikely, however, that a given ion should be mandatory for allergenicity in general.

Antigenic and allergenic determinants

One may ask if the IgE immune system reacts selectively to particular structural

arrangements (allergenic determinants) which differ from those acting as antigenic determinants in other immune systems. A number of studies investigating the structure of antigenic sites due to the primary structure of synthetic polypeptide chains reacting in the IgG system have been summarized by Gill (7).

The size of the antigenic (for IgG) site is generally 5–6 amino acid residues in a cluster, but can vary from a minimum of 2–3 residues to a maximum of 9 residues. Speaking in terms of metric measures, this means structures of $3.5 \times 1 \times 0.5$ nm or $2.5 \times 1 \times 1$ nm. There is as yet no comparable information about the composition and size of an allergenic determinant. However, work is in progress which may help to elucidate this.

The allergenic molecules of codfish parvalbumin have undergone fragmentations of different kinds. Several fragments have been analysed with respect to sequence and allergenic activity. The highest degree of allergenic activity is found in a peptide from residue 20–44, but even smaller peptides have been shown to be active when reacting with IgE antibodies *in vitro* and *in vivo*. However, with such small peptides the otherwise almost perfect correlation between *in vivo* and *in vitro* tests for reactions between allergen and IgE antibodies is diminished. This may indicate that aggregation or polymerization may occur in the biological system.

The major codfish allergen remains active even when treated with 8M urea which makes it unfold or attain a random coil form. This observation indicates that the allergenic determinant in question may be sequential. However, two reservations illustrating the difficulties encountered in this kind of research have to be made in this connection. Even when the allergenicity (antigenicity) remains after denaturation this may have been due to some residual folded structure in a sufficient number of molecules. Furthermore, even when the protein molecule is denatured, showing all necessary

evidence that it is unfolded in the laboratory (*in vitro*), the material may regain its original folded form as soon as it is introduced into the biological system. The antibodies against the native conformation may have induced the flexible, denatured polypeptide chain to refold into its native structure (8). There is no method available to control this in the skin.

Provided that sequential determinants are involved in the biological reaction, the complete amino acid sequence of the major allergen (Allergen M) from codfish provides enough substance to allow speculation about the nature and characteristics of the allergenic determinants. Certain features of the molecule may serve as theoretical allergenic determinant models to illustrate the kind of structure that may be involved in a sequential allergenic determinant (3).

The stereochemical characteristics of the allergenic determinant may be made up of a number of critical amino acid side chains which are kept at a critical distance from each other by other amino acid residues, the latter representing only an inert framework. Assuming this, the molecule may contain a total of six or more sites which theoretically could represent repetitive allergenic determinants. For instance, the critical site may be made up of two closely connected carboxylic side chains (Asp + Glu or Asp + Asp) kept at a critical distance from the basic amino of Lys by an amino acid residue, the nature of which is indifferent. The validity of such hypothetical models remains to be proven or dismissed through experiments with synthesized peptides in different forms. So far, a synthetic tetrapeptide (H – Asp – Glu – Leu – Lys – OH) and a synthetic octapeptide (H – Ala – Phe – Ser – Ala – Asp – Glu – Leu – Lys – OH) have been tested and shown not to interact with the particular IgE antibodies to Allergen M. This may cast suspicion on other parts of the molecule or shed doubt on the assumption that sequential allergenic determinants are important in Allergen M.

Much further work is needed for the

identification and characterization of an allergenic determinant. If successful, the availability of synthetic representatives of allergenic determinants may become important tools for basic studies in allergenicity and may pave the way for new therapeutic approaches.

Conclusion

The first steps have been taken toward elucidation of the molecular basis of allergenicity and the identification and possible synthetization of allergenic determinants, but there is still a long way to go. It is anticipated that further achievements in this field may reveal a high order of allergenic determinant individuality rather than common traits as regards physiochemistry of allergenic molecules, apart from being proteins or protein bound substances with molecular weights below 70,000 which react with specific antibodies of the immunoglobulin E class. The molecule in question must be bivalent or carry more allergenic determinants easily accessible for the IgE molecules. Furthermore, the accessible allergenic determinants must have an optimal spacing in order to react with the IgE antibodies on the mast cell surface.

It remains to be proven whether viral or bacterial components or carbohydrate molecules act as allergens in the IgE system.

Until several allergenic determinants have been identified and compared it is not possible to point out any other common characteristic which makes an allergen an allergen. It is conceivable that allergenicity is just antigenicity reflected by a special immunological system. Common characteristics are more likely to be expressed in terms of the allergic individuals, and depend on the genetic predisposition of the host and a number of immunological and non-immunological host factors regarding type, degree and duration of the particular exposure. With increasing immunogenicity of the molecules at the site of immunological response, the influence of subtle genetic

control on the specific immune response probably becomes less strict. More people are likely to become allergic to such molecules provided they belong to the IgE-responding proportion of the population and are sufficiently exposed to the substance in question.

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