

Discovery and development of IgE assays

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Introduction

Although the first cases of pollen-induced hay fever were described in the early 19th century it was not until 100 years later that a connection to a serum factor, later called reagin, was realized. In the year of 1919 a case of asthma caused by allergy to horse dander passively transferred by blood transfusion was reported [1] and a couple of years later Prausnitz & Küstner [2] performed their famous passive transfer of a positive skin test, later called the PK test. However, despite great efforts during the next 40 years very little progress was achieved with regard to isolation and characterization of the reagin. Now in retrospect, it is easy to understand why. The extremely low concentration of the allergy-mediating factor and also its great lability as far as cell-surface binding is concerned obscured most experimental data. In a textbook on immunological diseases printed in 1965, it was speculated that the reagin activity 'is not a single, indivisible molecular species but is present in allergic sera in the form of labile complexes' [3] and that it 'differ radically from immune antibodies' [3]. Since the activity was heat sensitive it was assumed that its biological function was highly dependent on conformational structures.

The underestimation of the extreme sensitivity of a reagin-mediated histamine release reaction is the most likely explanation for the report in 1964 by Ishizaka *et al.* [4], in which they finally proved that the reagin was an antibody, and this antibody belonged to the γ A immunoglobulin class. Obviously, their Fc α preparation was contaminated with trace amounts of IgE that was enough to cause the biological reaction but that could not be detected by the protein assays or be appreciated by the immunological concepts available at that time. However, a couple of well-known facts did not support the γ A statement. When reagin activity was traced in fractions of serum separated by combinations of gel filtration and ion-exchange chromatography, it was possible to show that γ A/IgA behaved differently to the reagin activity. In addition, patients genetically deficient of IgA could have high titres of reagin activity. Thus, the search for the nature of the reagin continued.

A new immunoglobulin

The Ishizaka group in Denver, Colorado, continued their experiments using methods for protein separation combined with immunization of experimental animals. In 1966–67 they claimed to have an antiserum that could precipitate the reagin, resulting in a decrease in reagin activity [5,6] as well as binding to a mixture of reagin serum and an allergen trace-labelled with an isotope in autoradiography. This factor apparently did not belong to any of the four known immunoglobulin classes and was therefore postulated to represent a new immunoglobulin, provisionally called γ E-globulin. One reason for the name was that when injected into human skin the fraction caused erythema, a reaction that, ironically, is not IgE-mediated. Despite hard efforts they never succeeded in purifying the γ E-globulin, which is not surprising considering its extremely low concentration.

Independently of this work and for quite other reasons, Bennich & Johansson, in Uppsala, Sweden, in 1965 detected an atypical myeloma protein that was found to share the physicochemical properties characteristic of the reagin [7]. Using at that time highly sophisticated, radioimmunoassays, a normal counter part, provisionally called IgND, could be detected in serum of healthy individuals [8] and it was found that patients with allergic asthma had on average a sixfold higher concentration of IgND than normals or patients with non-allergic asthma (Fig. 1) [9]. In collaboration with L. Wide, Uppsala, a radioimmunoassay, the radioallergo-sorbent test (RAST), was developed capable of detecting IgND antibodies to allergen that correlated well with skin testing [10]. In collaboration with D. R. Stanworth, Birmingham, UK, it was shown that the PK reaction in humans could be blocked in a dose-dependent way with isolated IgND [11] or Fc fragments of the ND protein [12].

In 1967 reagents were exchanged between the two laboratories and it was found that antiserum to γ E-globulin reacted with isolated ND protein and that purified ND protein could block the reaction of anti- γ E-globulin in a biological test system for reagin activity. These results, as well as immunological reagent including purified ND protein were submitted to the WHO immunoglobulin reference centre in Lausanne, Switzerland. At a workshop in Lausanne in February 1968, in which the main researchers from

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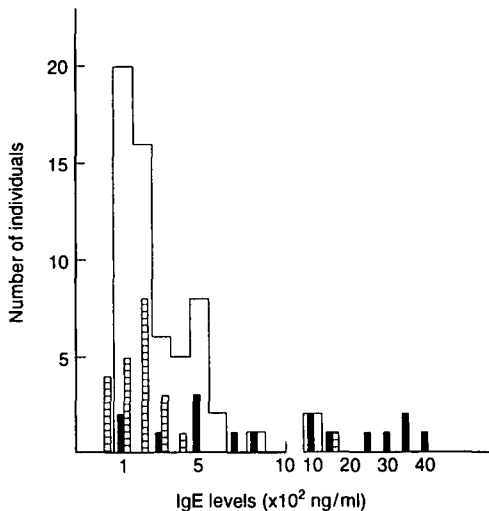


Fig. 1. The distribution of total IgND (IgE) levels in serum of patients with extrinsic, atopic asthma (solid bars) compared with patients with intrinsic, non-allergic asthma (hatched bars) and normal, healthy controls (solid line). Data from ref. 9.

the two groups participated, it was finally agreed that there were enough data available on the structure and antigenicity of the material to allow the declaration of a new immunoglobulin, which was called IgE [13]. In addition its relation to the reaginic activity was confirmed.

The clinical impact of IgE

Despite excellent work at many centres in the world, allergology in the mid-1960s was still a clinical speciality with a questionable reputation. In the absence of solid facts about mechanisms, the field was open for speculations. Allergy diagnosis was mostly based on intradermal skin tests and the allergen extracts used at that time were far from standardized. In this situation arguments between clinical centres or active research groups on reported data were strong and wild. It was almost a regular phenomenon that findings reported from one place could not be reproduced in another. The solution to the more than 40-year-old riddle of the nature of reaginic activity was a solid piece of science that certainly improved the standard and reputation of allergology.

Although theories and science in the late 1960s indicated that the reaginic activity through reaction with inflammatory cells stimulated the release of various vasoactive mediators of importance for the clinical symptoms and signs, only access to IgE allowed experimental, immunochemical studies *in vitro*. The research activity in this field increased enormously and scientists from areas other than classical allergy became interested in the field and fed into it knowledge of great importance. One example is the characterization of one of those mediators,

SRS-A, which was presented by biochemists initially without any interest in allergy as such. The discovery of the leukotrienes was in 1982 honoured by a Nobel prize to Sune Bergström, Bengt Samuelsson and John R. Vane. Today we have fairly good knowledge about the various steps in the allergic reaction, starting with contact with a protein allergen in the environment leading to an allergic inflammation that will cause sustained symptoms from mucosal membranes and skin. It is quite clear that each step in this chain of reactions is under genetic control and therefore the search for a single allergy gene seems a bit dead-endish.

One immediate benefit of the availability of IgE and the development of tests for IgE antibody was the possibility to analyse and standardize allergen preparations. It was found early on that labeling an allergen extract in w/v or PNU/ml was no guarantee for a given allergen potency. In fact, batches of extracts of simple allergen sources, such as pollen, from one single producer regularly varied in potency by a factor of 100; for moulds differences up to 10 000 were reported. Many extracts, e.g. bee and wasp allergens, did not contain any detectable allergen at all. Despite some difficulties for the authorities in the beginning to understand the difference between protein and antigen as measured by conventional protein or immunoassays on one hand and allergen defined as antigen reacting with IgE antibody on the other hand, the quality of the allergen extracts available improved quickly. However, still well standardized and characterized extracts of only approximately 20 allergens are available on the market. The next step in development is now being taken. Recombinant products of major allergens are continuously being used [14], today between 100 and 200 different products are available, with the aim to secure a reproducible production of allergen without any contamination of non-allergen material. The first allergen peptide products supposed to induce T-cell mediated tolerance are presently undergoing clinical trials [15]. Whether they will lead to a significant improvement of allergen-specific immunotherapy is still too early to say.

When specific antisera to IgE became available it was possible to develop *in vitro* tests for measurement of IgE antibodies to allergen. Results of the radioallergosorbent test (RAST), published in 1967 [10], showed a very good correlation between the presence of IgE antibody in serum and positive skin and provocation tests, as well as symptoms of allergy. Although a laboratory test for allergy was met with some scepticism by allergologists in the beginning, it is now an established technique for allergy diagnosis. Despite technical improvements the principal of the original RAST test dominates the test procedures used today. Other types of solid phases for the allergen, as well as alternatives to radioactivity as the

signal for the anti-IgE tracer antibody, have been introduced. Today approximately 400 different allergens are available for RAST-based allergy diagnosis. In addition to classical pollen, dander and food allergens, drugs, occupational chemicals and recombinant allergens are available. The general availability of the *in vitro* test to doctors, specialists or non-specialists in allergology, as well as the high reproducibility of the test result, has greatly improved the quality of allergy diagnosis in general.

The allergy dilemma

It might look like a paradox but the more we have learnt about the mechanisms of allergy the more the prevalence has increased. Epidemiological investigations from the industrialized world indicate prevalence figures for atopic allergy in the order of 30–40%. The reason for this increase is not completely understood but it is likely to be multifactorial and it has something to do with the high standard of living that characterizes the industrialized countries. Air pollution, especially cigarette smoke, is likely to be one important factor but other factors mentioned are car exhaust, inefficient ventilation of houses and changes in feeding patterns and infection panorama in early childhood. Based on a 30% prevalence it can be calculated that today, in Europe and the USA, there is approximately one specialist in allergology per 10 000–100 000 patients with allergy. Obviously, to encourage patients with allergy and non-allergic hypersensitivity to go to a specialist will not solve the problem. Using the objective laboratory tests for atopy and specific allergy it should be possible for non-specialists to handle a large proportion of the patients with rather simple symptoms of allergy or hypersensitivity. Using a determination of total IgE in serum and an allergen supermix test in RAST, more than 90% of patients with symptoms can be accurately identified as being atopic individuals and thus likely suffering from atopic allergy. It should then be possible to teach the non-specialists to use various combinations of mixed allergens in a second step to identify in a cost-benefit way the suspected allergen or allergens. These patients can, in most cases, be treated efficiently with allergen avoidance supported by the improved drugs available today.

Complicated cases, such as those with severe asthma or symptoms from many organs, or when a specific but rare sensitization has to be identified, are recommended to be referred to a specialist. They are likely to use, in addition to *in vitro* tests, skin testing and provocation tests in the affected organ in their diagnostic workup. It is also possible that they will start allergen-specific immunotherapy. Thus, by increasing the knowledge about allergic diseases among non-allergy specialists and taking advantage of the reproducible and objective laboratory tests, it should be possible

to design a health care system for allergy which will allow every allergic individual a good care.

Conclusions

During the 30 years that IgE has been known the knowledge about allergy has improved immensely. We have now available reliable procedures for allergy diagnosis, standardized allergen preparations for diagnosis and treatment, an improved knowledge about mechanisms behind allergy and tests to monitor the efficiency of therapeutic procedures. However, despite this increase in our knowledge the prevalence of allergic diseases is still increasing. More efforts must be dedicated to the understanding of why an atopic individual is sensitized, how this sensitization can be avoided by primary prevention, and hopefully to find a way to interfere and turn off or at least dampen the harmful effect of this sensitization. Allergy is a serious problem for hundreds of millions of people and no effort should be spared to improve their quality of life.

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