

The discovery of IgE

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To the protein chemist intent on explaining biologic activity of immunoglobulins in structural terms, IgE poses a formidable but rewarding challenge. I have been privileged to work on this topic for the last 37 years, having previously undertaken what has come to be recognized as the first serious attempt (18) to characterize chemically a major inhalant allergen (from horse dander). Consequently, I was in a key position to witness and participate in the exciting and sometimes dramatic events which culminated in the discovery of IgE.

As we look back on those early days, it is difficult to imagine now that so much progress could have been made since on the characterization of anaphylactic antibodies. At that time, they were referred to by the noncommittal term "reagin" (coined by Coca and Grove in 1925). And, as I wrote in a review article (20), as late as 1963, there was still doubt about their antibody status. Unlike antibodies of the then known immunoglobulin classes, they failed to give a precipitin reaction when incubated with a specific antigen (allergen), and they appeared not to fix complement *in vitro*.

Admittedly, Von Pirquet had expressed (as early as 1906) a belief that an antibody-antigen reaction was the basis of allergic phenomena. But it was not until the pioneering work of Prausnitz (1921) (16) in Germany that a candidate antibody was demonstrated to be present in the sera of allergic persons and to be capable of transferring immediate hyper-

sensitivity to the skin of normal human recipients. For the next 40 years or more, the Prausnitz-Küstner (P-K) test, as it came to be called, offered the only means of assaying reagin (reaginic antibody) activity.

Even in the pre-AIDS era, there was still the risk of transferring the hepatitis virus through blood serum, and, inevitably, this was a tremendous handicap in attempts to characterize this most elusive of antibodies. As far as my own early work in this area was concerned, there was an additional constraint because the then head of the department of experimental pathology at Birmingham University (the late John Squire) insisted that all P-K tests be performed on him personally (on his back) with serum (or reagin-rich fractions) from just one donor ("PR"), a member of our technical staff who was very allergic to horses and who had no clinical history of hepatitis. As P-K test sites are refractory for about 1 month afterwards, and as professors tend to spend substantial periods of time outside their departments, our opportunities for assaying reaginic antibody activity were somewhat restricted at that time.

Early characterization of reagents

Despite such limitations, it was from P-K testing that the first insight was obtained into the biologic and chemical properties of reaginic antibodies. Thus it was revealed that their cytophilic activity is de-

stroyed by heating allergic sera for 1 h at 56°C, that they fail to cross the placenta (to the advantage of the offspring of allergic mothers), and that they come down in the same fraction as γ -globulin on salt precipitation of allergic sera.

During a year spent working at New York University, I took advantage, with Bill Kuhns, of the availability of healthy medical student recipients to look in detail into the way various factors influence the accuracy of the P-K test (27). This led to an improvement in the quantitative capability of what was a very sensitive, albeit *in vivo*, method of measuring reaginic antibody activity. But the extremely low level of reagin (rarely more than a few micrograms per millilitre in sera of even the most intensely allergic persons) thwarted any hope of establishing in which peak it was located during free solution electrophoresis or analytic ultracentrifugation, as the limits of detection of the Schlieren optical systems employed in such apparatuses were around 3000 times too insensitive to reveal reaginic antibody as a distinctive peak.

As I pointed out at the time (20), the attempted isolation and physicochemical characterization of reagins was handicapped by difficulties inherent in the purification of a trace serum constituent, by the marked instability of the reagin molecule when separated from other serum protein constituents, and by the inadequacies of the *in vivo* method of assaying antibody activity. Another major technical disadvantage in our early attempts to characterize reaginic antibodies physicochemically, as discussed in detail later (23), was that electrophoresis or ultracentrifugation of protein mixtures (such as serum and plasma) in free solution does not lead to an absolute separation of the different protein constituents; and even our use of partition ultracentrifuge cells in an attempt to overcome this problem, and thereby preferentially separate reagin-containing fractions, failed.

The first significant advance came with the development of zone-fractionation (e.g. ultracentrifugation and electrophoresis) techniques; their advantages were the greater resolution and stabilization of minor protein constituents thereby afforded, and the possibility of associating biologic activity with a readily identifiable protein constituent of a particular fraction. Application of these new procedures provided the first information that reagins moved in the "fast- γ " (i.e. " γ 1") region on zone electrophoresis, and in the 7s region on ultracentrifugation in a density gradient. We obtained the latter information by initially performing relatively crude zone-centrifugation runs in buffered sucrose gradients (18), involving the slicing of the lusteroid tubes into a limited number of fractions (i.e. four) for P-K assay. Nevertheless, the results thus obtained discounted earlier claims that reagins were macroglob-

ulin antibodies, while other more refined studies on the sedimentation of allergic sera in density gradients (1), and of its gel filtration through Sephadex S-200 (31), revealed that reagins move in an unusual position, as compared with the then known immunoglobulins (7s and 19s γ -globulins), i.e. slightly ahead of the 7s component.

Attempts to associate reagin with a known class of immunoglobulin

Another important methodological advance made around this time was to have a decisive impact on attempts to characterize reaginic antibodies. As a result of work undertaken with Ed Franklin in New York (6), antisera were raised against paraproteins which were capable for the first time of distinguishing the then known classes of immunoglobulin (IgG, IgM, and IgA) immunochemically. Not surprisingly, this new tool was exploited in attempts to confirm that reagins were indeed antibodies and to associate them with a particular class of immunoglobulin.

An attractive idea put forward at the time (8) was that reagins belonged to a recently discovered, third class of immunoglobulin (IgA, originally referred to as " β 2A"); an antibody of this type was found to be prevalent in nasal fluid, saliva, and other secretions, where it was thought to be involved in the body's "front-line" defences. Furthermore, the removal of this immunoglobulin from allergic sera by absorption with specific (sheep) anti-IgA globulin appeared to remove also their reagin activity as measured by P-K testing (5). Moreover, it was shown that P-K activity could be blocked by normal human IgA (9) and by purified human α -chain (10) when such preparations were injected into a normal recipient's skin before passive sensitization with allergic serum, or when they were administered simultaneously with the transferred reagin.

What concerned me about these claims that reagin was a member of the IgA class of immunoglobulin was my observation (21) that chromatographic fractionation of allergic serum on a diethylaminoethyl(DEAE)-cellulose column resulted in the elution of maximal reaginic activity *far ahead* of the IgA peak. Soon after these data were published, Kimi and Terry Ishizaka visited my laboratory, on which occasion I hotly contested their claim that reagin belonged to the IgA class of immunoglobulin. This encouraged them to look again at this question with the result that they developed an improved multistep procedure for the fractionation of potent sera from ragweed-sensitive persons, and this produced a highly purified reagin fraction. Production of a specific antiserum against this P-K-active allergic serum γ -globulin constituent suggested that reagin was not, after all, of the IgA class, but, rather,

was antigenically distinct from the three then known human immunoglobulin classes (11). This was based on the observations that the reaginic activity of sera from ragweed-sensitive patients could be eliminated after precipitation with the rabbit antiserum, which did not contain antibodies against the three major immunoglobulin classes (IgG, IgM, and IgA), and that, by radioimmunodiffusion and radioimmuno-electrophoresis, an antibody against the principal allergenic constituent ("antigen E") of ragweed pollen extract could be detected in the γ 1-globulin precipitin band formed on interaction of the rabbit antiserum with reagin-containing allergic serum fractions.

Around that time, a fourth human immunoglobulin class (IgD) was discovered (17). We were unable, however, in preliminary studies to demonstrate removal of P-K activity from allergic sera by absorption with antibodies directed against this new class of immunoglobulin.

Discovery of Ig"ND"

It was against this background that Bennich and Johansson discovered in Uppsala a paraprotein, in the serum of a myeloma patient ("ND") with plasma cell leukaemia, which lacked antigenic determinants of the four then known immunoglobulin heavy chains (γ , μ , α , and δ). Intriguingly, this unusual paraprotein showed remarkably similar physicochemical properties to reagin (referred to previously); that is, it moved in the "fast- γ " position on zone electrophoresis, it eluted just ahead of the 7s fraction on gel filtration of allergic serum on Sephadex G200, and it possessed a sedimentation coefficient much closer to 8s than 7s! Could, therefore, this atypical myeloma protein (initially designated Ig"ND") be the product of plasma cells producing a hitherto unrecognized class of immunoglobulin of which reaginic antibodies were representative?

This idea had occurred to Mac Turner (currently European editor of the *Journal of Immunological Methods*), who happened to be a visiting scientist in the Uppsala laboratory at the time, prompting him to suggest that a sample of the Ig"ND" myeloma protein be sent to my laboratory in Birmingham for testing in a passive leucocyte assay (i.e. a modification of a method first described (32) by Van Arsdel & Sells in 1963), which we were employing at the time to measure reagin activity *in vitro*. But this was not the easiest of tests to perform in an inhibition mode, and our attempts to show that the Ig"ND" paraprotein blocked the sensitization of normal human leucocytes (basophils) by reaginic antibody gave equivocal results. At a symposium of the British Society for Immunology held in London in spring 1967, I reported these findings briefly, in a talk on

immunoglobulin Fc effector activities. The chairman of the symposium, the late John Humphrey, asked whether we had done the obvious experiment; namely, test whether the new myeloma protein was capable of blocking the sensitization of a normal person's skin by reaginic antibody in a P-K assay. I answered that this was something which we would dearly like to do but we wondered who would be willing to undergo the myeloma protein injection. He volunteered immediately because he was preparing (with the late Bob White) a new edition of his book *Immunology for medical students*, and he felt that it was important to report the outcome of such a key experiment in that popular publication.

Five days later the experiment had been completed, and we were able to report (28) that, when introduced to the intradermal site 18 h earlier, the myeloma protein Ig"ND" (at a concentration of 300–3000 ng) was able to bring about complete blockage of the subsequent sensitization of that site by reaginic antibody in the serum of a horse-dander-sensitive person, as revealed by the absence of a wheal and flare response to later (24 h) challenge with horse dander allergen. We adopted the same approach later (29) to demonstrate that, of the various chemical and proteolytic cleavage fragments of the Ig"ND" protein tested, only the Fc fragment inhibited the P-K test in a manner similar to the whole myeloma protein, providing the first experimental evidence that IgE antibodies bind to Fc receptors on skin mast cells, as had been suspected for some time (20).

At a Nobel symposium held in Uppsala in June 1967, Johansson & Bennich (14) reported convincing evidence that the new myeloma protein Ig"ND" was indeed representative of a new class of immunoglobulin and, significantly, that it was possible to show by radioimmunoassay (employing a polyvalent antiserum directed specifically against the paraprotein) that a similar immunoglobulin was detectable at elevated levels in the sera of patients with allergic asthma but not in intrinsic asthmatic patients' sera (12). These findings suggested that the new class of immunoglobulin (Ig"ND") was closely related, if not identical, to "reagin".

But it was the results of our inhibition P-K tests (referred to above) which provided the final piece to the long-standing puzzle of the nature of reaginic antibody, which the work of Ishizaka and associates suggested might be representative of the unique class of immunoglobulin which Johansson and Bennich had discovered in Sweden, and had shown to be antigenically similar to reagin. Consequently, the investigators from our three laboratories (Fig. 1) who gathered in Lausanne in February 1968, under the auspices of the WHO Immunoglobulin Reference Laboratory, concluded that a new (fifth) class of

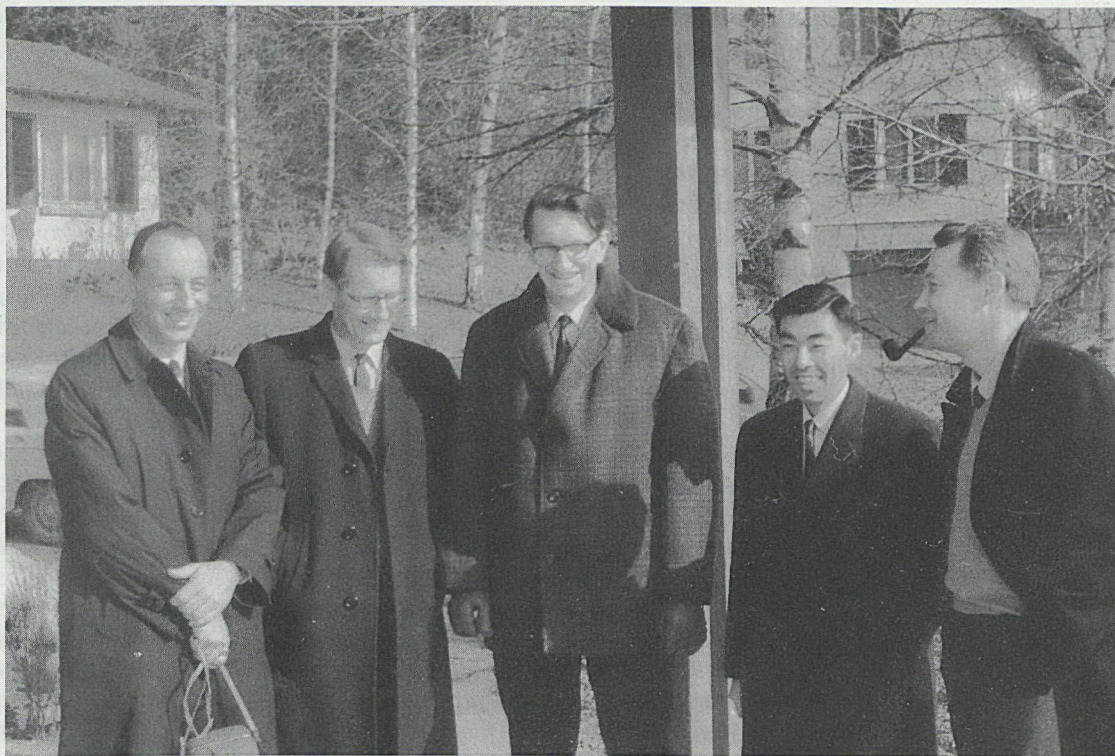


Fig. 1. A break in the discussions on IgE in Lausanne in February 1967. From left to right: Terry, Rowe, Stanworth, Ishizaka, Bennich. Behind the camera, SGO Johansson.

immunoglobulin had been discovered, of which reaginic antibodies were representative, and that this should be designated IgE (2). This article is appearing on the 25th anniversary, to the month, of that momentous meeting.

Conclusion

It is impossible to overrate the impact that these developments have had on the laboratory and clinical investigation of immediate hypersensitivity of the asthma and hay fever type.

Unlike the major classes of immunoglobulin (IgG, IgM, IgA), IgE is present in serum (even of the most allergic patient) in minuscule amounts. Thus, as predicted in 1971 (22), the availability of relatively huge quantities of a monoclonal (paraprotein) form, followed later by the discovery of additional IgE myeloma proteins in other parts of the world and their *in vitro* production by cultured B-cell lines, has (as in the earlier case of the availability of myeloma proteins of the other immunoglobulin classes) provided the key to the complete primary structural analysis of the reaginic antibody. More recently, the use of synthetic peptides representative of various IgE Fc sequences and antibodies against these as structural probes is beginning to reveal important new insights into the molecular pathology of this intriguing im-

munoglobulin (3, 4, 24, 26). Moreover, one such peptide thought to be representative of a mast cell trigger site within the CH4 domain of IgE is now being developed as a novel form of vaccine for the treatment of IgE-mediated allergies (25, 30).

Another major "spin off" from the discovery of IgE has been, of course, the development of *in vitro* methods of detecting and measuring reaginic antibodies (33); these have found extensive application in both clinical and experimental allergy. Although it was eventually shown (15) that reagin activity could be determined in human allergic serum by PCA testing in higher subhuman primates, this type of assay would never have become widely available and would certainly not have been accessible to the routine clinical allergy laboratory.

Now we have reached the exciting stage where recombinant DNA technology is being used to delve even more deeply into the molecular pathology of IgE, offering the hope of developing innovative, and more selective, methods of treating immediate-type allergies. Meanwhile the application by biophysicists of techniques such as X-ray solution scattering is beginning to throw light on the three-dimensional structure of IgE and how it interacts with the high-affinity Fc ϵ RI receptor on mast cells (7). It is safe to bet that we would have progressed a much shorter distance along this road, but for the astute observa-

tions of Gunnar Johansson and Hans Bennich in Uppsala, which resulted in the discovery of the first myeloma form of a unique class of immunoglobulin, of which reagin turned out to be representative!

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