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Science behind the discovery of IgE

The race for identification of the reagin or serum factor that could spontaneously sensitize skin and mediate a positive immediate-type skin reaction began in 1921 with the report of Prausnitz and Kustner.¹ In their classic experiment, Prausnitz passively sensitized the skin in his forearm to fish by injecting serum from his coworker allergic to fish, Kustner. The following day, Prausnitz had an immediate-type wheal and flare, or so-called *P-K reaction*, after injection of fish extract into the same site in the skin. A detailed account of this exciting discovery has been eloquently presented in the Allergy Archives of the Journal,² and it is not further discussed here.

Once human serum was known to carry the reagin, many investigators attempted to identify it over a period of more than 40 years. Eventually, 2 groups took advantage of newly developed analytical methods for serum protein fractionation and the availability of IgE myeloma proteins and adsorbed IgE (reagin- γ E-ND) specific antisera. They would independently arrive at the conclusion in 1967 that the reagin was in fact a unique immunoglobulin class.³ This report reviews the scientific observations that led them to the discovery that IgE is a unique human immunoglobulin class and the elusive reagin or skin-sensitizing factor in serum. Once the science has been discussed, the report provides a personal reflection on the significance that the discovery of IgE has played in improving the clinical diagnosis and management of patients with

allergy and facilitating basic research involved in studying mechanisms of human allergic disease.

IGE: A NEW CLASS OF HUMAN IMMUNOGLOBULIN

By 1960, several analytical techniques including ammonium sulfate precipitation, Cohn fractionation with ethanol, zone electrophoresis, anion exchange resin chromatography, and sucrose density gradient ultracentrifugation had been used by researchers to conclude that reaginic antibodies were different from IgG (γ G) and IgM (γ M). Many of the key scientific observations leading to this conclusion are listed in Table I. After identification of IgA (γ A, originally known initially as β 2a globulin) in 1959, many researchers began examining the potential association between human IgA and reaginic activity in serum. The γ A fraction of human atopic sera was initially shown to have skin-sensitizing activity, and γ A globulin but not γ G or γ M from normal serum was able to block passive sensitization of human skin with reagin. These and other experimental data (Table I) lead some scientists to conclude that reaginic activity was associated with IgA. Others, however, remained skeptical about this conclusion because there was a possibility of impurities in the IgA preparations containing the reaginic activity that might not have been detected by the immunochemical methods available at the time. The sensitivity of the P-K reaction in human beings was thought to be comparable with that of the passive cutaneous anaphylaxis (PCA) reaction in guinea pigs, in which 0.03 to 0.1 μ g nitrogen/mL antibody was required for a positive reaction. However, because some reaginic sera with a γ A concentration of 0.3 mg nitrogen/mL titrated out to 1:10,000, this would have meant that all of the γ A in these sera would have had to be specific for a single allergen specificity (which was considered unlikely). To shed further doubt on the association between reaginic activity and γ A, a serum was identified with reaginic activity that lacked any detectable γ A. Finally, human anti- γ A isoagglutinin studies indicated no detectable γ G, γ A, or γ M antibodies in sensitized skin.^{19,20}

By the mid-1960s, Drs Kimishige and Teruko Ishizaka had begun repeating some of their earlier experiments, this time by using more specific antisera for reagin and chromatographically separated fractions of human serum from individuals allergic to ragweed and egg. They observed that skin-sensitizing activity in the chromatographic fractions of these sera did not parallel γ A or γ G levels. Precipitation of γ G and γ A from these fractions with γ G and γ A specific antisera left reaginic activity in the supernatant, which was then precipitated out with anti- κ and anti- λ light chain antisera. Together, these results indicated that skin-sensitizing reagin was an antibody that was neither IgG nor IgA but possibly a unique protein having light chains of immunoglobulins.²¹ These results were

TABLE I. Selected historical events associated with the discovery of IgE as the reagin

Date	Observation	Method	References
1930-1937	Reaginic antibodies present in pseudoglobulin fraction rather than euglobulin fraction of human serum	Ammonium sulfate precipitation	4,5
1952	Reaginic activity concentration in fraction III containing principally β -globulin	Cohn ethanol-low temperature Fractionation	6
1954-1957	Maximum reaginic activity found in β to γ 1-globulin region	Zone electrophoresis	7-9
1957-1959	DEAE γ G immunoglobulin fraction usually lacked reaginic activity	Diethyl amino ethyl cellulose (DEAE) column chromatography	10,11
1961-1964	Reaginic activity found at 7.8 (average) between 7S (monomeric) and 19S (pentameric) fractions	Sedimentation coefficient by sucrose density gradient ultracentrifugation	12,13
1962-1964	Reports that γ A fraction of human atopic sera had skin-sensitizing activity	Immunoprecipitation	14-16
1963	γ A globulin but not γ G or γ M from normal serum blocked passive sensitization of human skin with reagin	P-K testing	17
1963	Ragweed-specific IgA (γ A) antibodies detected in sera of patient with allergy	Radioimmuno-electrophoresis	18
1964	Reaginic activity detected in human serum that lacked detectable γ A	Immunoprecipitation	19
1962	Human anti-A isoagglutinin studies indicated no γ G, γ A, or γ M antibodies in sensitized skin	Immunohistology	20
1966	Reaginic activity did not parallel IgG or IgA concentrations in fractions of serum from patients allergic to ragweed and egg processed with DEAE chromatography and Sephadex G-200 (Pharmacia, Uppsala, Sweden) gel filtration	P-K test, immunodiffusion	21
1966	Precipitation of γ A and γ D from reagin-rich serum fractions with added γ A isoagglutinin resulted in reaginic activity remaining in supernatant and indication that the reaginic antibody is not associated with γ G, γ A, γ M, or γ D globulins; labeled γ E-globulin	Chromatography, precipitation, electrophoresis, immunodiffusion, hemagglutination, P-K test	22-24
1996	Confirmation of lack of correlation between reaginic activity and γ G, γ A, or γ D concentrations in reaginic sera	Chromatography-P-K	27
1966-1967	Lack of association of reaginic activity with γ G2, γ G3, and γ G4, supporting conclusion that reaginic antibody belongs to a unique immunoglobulin class not yet described	Chromatography, precipitation, electrophoresis, immunodiffusion	24, 28
1967	Preparation of γ E antiserum and use of radioimmunodiffusion to show that γ E precipitin bands with serum from individuals allergic to ragweed bind radioiodinated antigen E (Amb a 1)	Radioimmunodiffusion	28-30
1967	Identification and physicochemical characterization of γ E-myeloma (ND)	Chromatography, precipitation, electrophoresis, immunodiffusion radioimmunoassay	32-34

confirmed with a reaginic serum fraction in which γ A isoagglutinin was added to insure IgA precipitation. The Ishizakas then went on to show the sucrose sedimentation velocity of reaginic antibody was between the polymeric (10.5S) and monomeric (7S) forms of γ A. γ A isoagglutinin also exhibited a faster electrophoretic mobility than reaginic antibodies.^{22,23} Multiple groups were able to confirm that γ A precipitin bands from ragweed-sensitive sera bound radioactive ragweed allergen and that anti-ragweed γ A antibody was precipitated by anti- γ A antibody, but it left essentially all of the reaginic activity in the supernatant.²⁴⁻²⁶ Only few groups such as Fireman et al¹⁶ continued to believe that reaginic activity was associated with γ A because their γ A antisera precipitated the reaginic activity. In retrospect, the antiserum used by this group for precipitation must have contained antibodies specific for IgE as a result of

a low level of IgE contaminant in the γ A preparation used for rabbit immunization.

To prove that reaginic antibodies belonged to a distinct immunoglobulin class, the Ishizaka group prepared rabbit antibodies to the reaginic-rich fractions of serum from ragweed-sensitized individuals. The antisera were then adsorbed against normal human γ G and myeloma γ A and γ D proteins to render them specific for reagin. The reagin-rich supernatants from γ A and γ G precipitation studies produced a distinct precipitin line with this reagin-specific rabbit antiserum. The precipitin bands were also shown to bind radioiodinated purified ragweed antigen E (Amb a 1). The reagin precipitin line did not contain γ G, γ A, γ M, or γ D, and the Ishizakas tentatively designated the antibody γ E-globulin.^{24,29} Confirmation of γ E's association with reaginic activity continued with repeat studies using fractionated reaginic sera from

TABLE II. Physicochemical properties of human immunoglobulins

Property	γ G	γ M	γ A	γ D	γ E	Myeloma ND	Reagin
Sedimentation coefficient $S_{20\text{ w}}$	6.6	18	6.6	7.0	8.0	8.2	~8.0
Electrophoretic mobility	γ 1- γ 2	γ 1	γ 1	γ 1	γ 1	γ 1	γ 1
Molecular weight	150,000	900,000	180,000	NA	NA	200,000	NA
Carbohydrate %	2.9	11.8	7.5	NA	NA	10.7	NA

Modified from J Allergy Clin Immunol, Volume 42, Ishizaka K, Ishizaka T, "Human reaginic antibodies and immunoglobulin E," Pages 330-63, Copyright 1968, with permission from the American Academy of Allergy, Asthma and Immunology.

multiple ragweed-sensitive patients. γ E-globulin specific antiserum was used in radioimmunoassay studies to show that precipitated antibody bands also bound radioiodinated purified antigen E.^{29,30} γ E-globulin was also shown to contain κ and λ light chains, and it was shown to not be associated with any of the subclasses of IgG or IgA.³¹

In 1967, Drs S.G.O. Johansson and Hans Bennich described an E myeloma protein (myeloma ND [after the patient's initials]) with physical and chemical properties that were similar to those of the γ E-globulin used in the Ishizakas' experiments.^{32,33} The anti-IgND serum specific for the Fc portion of the ND myeloma produced a single precipitin band (line of identity) in immunodiffusion studies. Anti-IgND also precipitated reagin activity from a ragweed-sensitive serum. The molecular weight of the IgND myeloma protein was slightly higher than that of γ G, and the monomeric form of γ A and its carbohydrate content was similar to γ M.³² By using a newly developed radioimmunosorbent test (RIST) that used IgND specific antiserum as a capture and labeled detection antibody, the Swedish group measured and reported the concentration of total IgND in sera of nonallergic (normal) individuals to range from 0.1 to 0.7 μ g/mL, with an average concentration of 0.33 μ g/mL.³⁴ This contrasted with IgG levels ranging from 7 to 15 mg/mL in the human serum. With the RIST assay, sera from atopic individuals with asthma and hay fever were shown to contain elevated levels of IgND.³⁵ Wide et al³⁶ then modified the RIST, replacing the solid phase anti-IgND with a solid phase allergen (allergosorbent) and using the same radiolabeled antihuman IgND as a detection antibody. The RAST was thus born as the first clinically useful assay for allergen-specific IgE antibody in human serum.³⁶

A new era in the diagnosis and research of allergic disease dawned with a World Health Organization (WHO) meeting in which the research findings of the 2 groups were merged. After a consensus that was arrived at the WHO International Reference Center for Immunoglobulins in Lausanne, Switzerland, in February 1968, the term *IgE* was accepted as that component in serum that carried reaginic activity.³⁷ IgE replaced other terminology such as *γ E-globulin*, *IgND*, and *reagin* that had been previously used in the literature to refer to skin-sensitizing antibodies present in serum of individuals with allergy. IgE was shown to have antigenic determinants in common with the other 4 human immunoglobulin classes (IgG, IgA, IgM, and IgD), and IgE from nonmyeloma sources was shown to contain both light chains (κ and λ). Studies with 2

IgE myeloma proteins (ND and PS) confirmed the molecular weights of the IgE heavy and light polypeptide chains as 75,500 and 22,500, respectively.³⁸ IgE's overall molecular weight was 180,000, and its sediment coefficient was shown to be 7.925 (Table II).³² The heat-labile nature of IgE was shown to be localized to the Fc region, and it correlated with structural changes within the C ϵ 3 and C ϵ 4 regions but not the C ϵ 2 region of the molecule.

SIGNIFICANCE OF IGE DISCOVERY FOR THE FIELD OF ALLERGY

The identification of IgE as the reagin has had a major effect on all aspects of the clinical diagnosis and treatment of patients with allergy, as well as on research investigations into the immunochemical pathways involved in the induction of effector cell degranulation and the elicitation of allergic symptoms.

Diagnosis and clinical treatment of patients with allergy

After acceptance of IgE as the reagin in 1968, the Swedish investigators moved rapidly to develop a clinically useful serological assay for total (RIST) and allergen-specific IgE antibody (RAST). For the first time, the diagnosis of human allergic disease could be accomplished by an *in vitro* alternative to skin testing that involved detection of allergen-specific IgE in serum. The first commercially available RAST for clinical laboratories used a paper disk allergosorbent to bind specific IgE antibody and radioiodinated antihuman IgE Fc was then used to detect bound IgE.³⁹ Bound radioactivity detected in the assay was translated into estimates of IgE and IgE antibody concentration by interpolation from a reference curve constructed with 5 dilutions of IgE antiserum reference serum. Total IgE results were reported in kilo international units per liter and specific IgE antibody levels in Phadebas relative units per milliliter. These results were often translated into arbitrarily chosen class groupings that were analogous to those being used to grade skin tests. This serological assay for identifying the presence of IgE antibody (sensitization to an allergen) was a useful diagnostic tool, especially for the evaluation of individuals with severe eczema or dermographism and children who were being investigated for allergies.

Over the 36 years since the 1968 WHO consensus meeting, many versions of IgE antibody assays have

come and gone. It was clear from a consensus meeting held by the National Committee on Clinical Laboratory Standards⁴⁰ that sufficient quantities of human serum containing IgE antibodies to each of the ~200 clinically important allergen specificities would not be available to calibrate each allergen-specific IgE antibody test with homologous standards. Thus, an alternative approach was adopted, the method of heterologous interpolation,⁴¹ in which allergen-specific IgE results were interpolated from a (heterologous) total serum IgE dose response curve. If the assay response (CPM-bound, OD, or fluorescent units) obtained for the total IgE calibration curve paralleled the responses obtained in the same assay with dilutions of test sera in the allergen-specific IgE antibody component of the assay, then the assay could be considered quantitative. Fortunately, this calibration scheme maintained parallelism, and the total IgE calibration curve has been traced in all clinically used IgE antibody assays to well-characterized human serum reference preparations with defined levels of total serum IgE.⁴²⁻⁴⁵ The quantitative nature especially of the commercially available third-generation allergen-specific IgE assays has allowed the identification of IgE antibody 95% confidence limits for some food allergen specificities. The consequence has been the option to avoid cumbersome and risky double-blind, placebo-controlled food challenges when assessing some children for the presence of clinical sensitivity to some allergic foods.^{46,47}

With regard to management of allergic disease, the identification of IgE as the reagin has led to development and licensing of anti-IgE (Omalizumab, Xolair [Genentech and Novartis, S. San Francisco, Calif]) therapy. Pretreatment total serum IgE levels are used to define the dose of Omalizumab to be administered. For traditional immunotherapy, pretreatment serological analyses for IgE antibody can aid in identifying the allergic sensitivities of patients with allergy so immunotherapy can be more effectively targeted. Changes in IgE antibody levels can also be used to assess the efficacy of treatment in patients with allergy receiving a combination of avoidance, pharmacotherapy, immunotherapy, and anti-IgE therapy. Finally, as more recombinant allergens are identified and purified, more precise characterization of the patient's IgE antibody specificity may allow more targeted therapy.⁴⁸

The competitive inhibition format of the RAST, and its modern nonisotopic counterparts such as the ImmunoCAP System (Pharmacia, Kalamazoo, Mich) and Immulite 2000 (Diagnostic Products Corporation, Los Angeles, Calif), have become important assays to allergen manufacturers and regulatory agencies for assessing allergenic potency of biological extracts and to the food industry for detecting and quantifying residual allergenic proteins in food products for purposes of labeling. More specifically, the power of IgE-based competitive inhibition immunoassays to quantify the presence and amount of allergens with

immunoreactive epitopes from egg (lysozyme) and soy and wheat has remained unsurpassed in comparison with total protein assays, antigen immunoassays, and PCR-based DNA assays.

Research on human allergic disease

Possibly nowhere has the discovery of IgE had such a large effect as on the investigator performing research on human allergic diseases. Selection of subjects with allergy for studies has been enhanced with quantitative total and allergen-specific IgE antibody assays. Often inclusion and exclusion criteria are set on the basis of the presence of certain IgE antibody specificities or levels of total serum IgE. IgE antibodies have been extensively used to study the presence of IgE and Fcε receptors on mast cell and basophil surfaces. Basophil mediator release assays have become a useful research and clinical tool for qualifying individuals who have discordant skin and serologic tests for IgE antibody. *In vitro* IgE-mediated activation of basophils has become a useful indicator of activation to identify better the biochemical pathways leading to *in vivo* mediator release. Even studies to identify genes that associate with allergy and asthma have used total and allergen-specific IgE antibody levels in serum to define probands and investigate associations between gene polymorphisms and allergic disease. The discovery of IgE has had sweeping effects on all aspects of research in the field of human allergic disease.

CONCLUSION

We owe a debt of gratitude to all those researchers, especially Drs Ishizaka, Johansson, and Bennich, who worked tirelessly with early immunochemical methods and often less than specific antisera to identify IgE as the reagin or skin-sensitizing antibody. Knowledge that IgE is the reagin that mediates allergic reactions has touched every aspect of our discipline. It has put a face on the elusive reagin that has plagued patients with allergy since the beginning of recorded time. Knowledge of its role in the immediate-type hypersensitivity reaction has led to improved diagnostic methods and enhanced clinical management. The discovery of IgE is that critical event in history that will have a lasting effect on the study of human allergic disease into our distant future.

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