

Landmark papers in our journal: Articles I to III of the series describing the discovery of IgE by the Ishizakas



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The discovery of IgE more than 50 years ago marks a turning point in modern allergology. With the biochemical and functional characterization of the unique antibody class responsible for immediate-type hypersensitivity reactions, an avalanche of research activities was initiated. These activities were not only in the field of modern (*in vitro*) allergy diagnostics but also in the arena of understanding the pathophysiology of immediate-type hypersensitivity reactions (both early-phase and late-phase responses) and the field of targeted therapies, cumulating in the development of mAbs against IgE.

The groundbreaking discovery of IgE by Kimishige and Teruko Ishizaka (the Ishizakas) is considered to have been first described in the 2 articles published in the *Journal of Immunology* in 1966.^{1,2} However, they were parts of a series of 6 articles,¹⁻⁶ all titled “physicochemical properties of reaginic antibody.”¹⁻⁶ The first 3 articles were published in the *Journal of Allergy* in 1966 and set the stage through a detailed biochemical and functional characterization of the so-called “reaginic factor.”³⁻⁵

Reagin, a serum factor that can cause a skin allergic reaction, had not been characterized for more than 40 years since its discovery in 1921. Then, by 1964, several groups of investigators reported that the reaginic activity in human sera was found in the IgA fraction but not the other immunoglobulin fractions. This reaginic activity was measured by using the Prausnitz-Küstner test, which at that time was the only method available to detect or quantitate allergen-specific substances associated with immediate hypersensitivity.⁷ The Ishizakas also published a similar report supporting this observation that reaginic activity belongs to the serum IgA fraction. However, the Ishizakas had also observed that the aggregated Fc portion of immunoglobulin is sufficient to induce an allergic reaction and were considering that the structure of the Fc portion might determine the functional nature of the immunoglobulin class. They found it illogical to accept that only a certain portion of IgA can induce a reaginic reaction,

Year	Discovery
1921	Identification of serum reaginic factor in an allergic patient (by Prausnitz and Küstner)
1963-1964	Reports of the reaginic factor as being indistinguishable from IgA by electrophoresis (by several investigators)
Mar. 1966	Paper I by the Ishizakas: Demonstration of the reaginic factor, which is neutralized with antibody against light chain of Immunoglobulin (Ig) but not that against IgG or IgA (ref. 3)
Jun. 1966	Paper II by the Ishizakas: First demonstration of reaginic factor, separable from IgA by electrophoresis (ref. 4; see Fig. 2)
Aug. 1966*	Paper III by the Ishizakas: Further purification of reaginic factor, as a minor serum component, which is not neutralized with antibodies against known Ig classes (ref. 5)
Jul. 1966	Paper IV by the Ishizakas: Successful production of antibody against the above purified reaginic factor, and demonstration of neutralizing effect of the antibody on skin allergic reaction (ref.1)
Nov. 1966	Paper V by the Ishizakas: Visualization of the reaginic factor bound to its specific antibody as a distinct precipitate band from known Ig classes using electrophoresis (ref.2)
Sep. 1967	Paper VI by the Ishizakas: Demonstration of IgE but not IgG/IgM as a heat-sensitive molecule (ref. 6)
Oct. 1967	Identification of new myeloma protein distinct from known immunoglobulin classes (by Johansson and Bennich; ref. 8)

FIG 1. The chronological chart regarding the discovery of IgE and the series of “physicochemical properties of reaginic antibody” articles by the Ishizakas. The first 3 “physicochemical properties of reaginic antibody” articles (colored boxes) were published in the *Journal of Allergy*: article I, “Association of reaginic activity with an immunoglobulin other than γ A- or γ G-globulin”³; article II, “Characteristic properties of reaginic antibody different from human γ A-isohemagglutinin and γ D-globulin”⁴; and article III, “Further studies on the reaginic antibody in gamma-A-globulin preparations.”⁵ The rest of the series article were published from the *Journal of Immunology*. *Although article III was published slightly after article IV, the date of submission was earlier than that for article IV.

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whereas the rest of the IgA can induce isohemagglutinin of blood group substances but not reaginic reactions.⁷ Then an observation (published by Loveless as an abstract in 1964) that reagin activity was present in a man who lacked IgA led them to re-examine their earlier finding.³ Thus the Ishizakas started the series of studies called “physicochemical properties of reaginic antibody” (Fig 1).³⁻⁶

In the first report³ in this series, they discovered that the skin-sensitizing antibody was associated with neither IgM nor IgA but with a unique protein containing light chains of immunoglobulin by making and using their specific antibodies. The Ishizakas also found that a very small amount of this unique

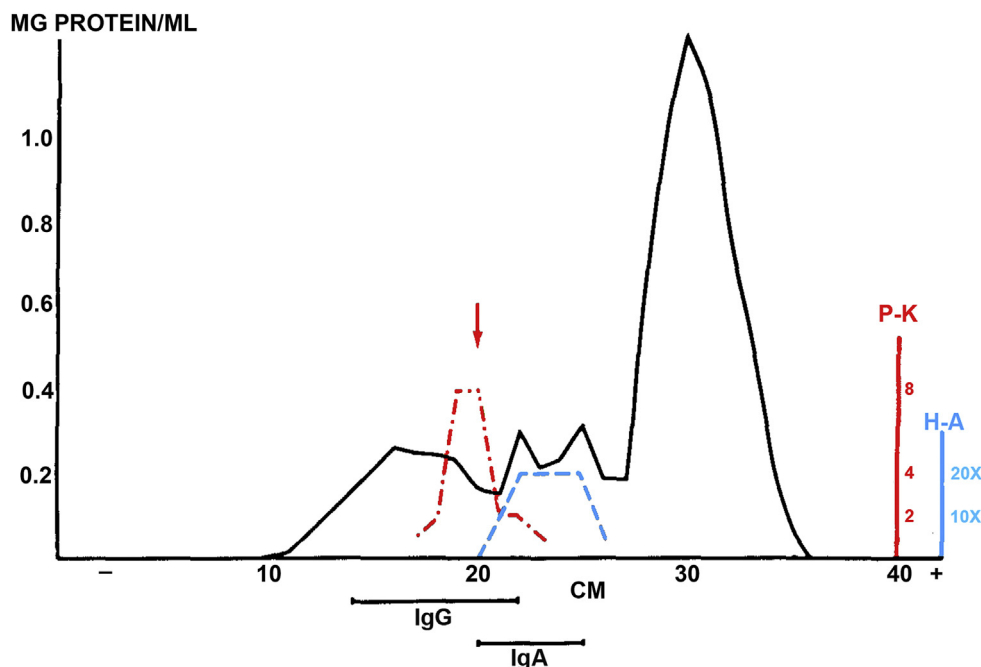


FIG 2. First demonstration of a carrier protein of reaginic antibody (IgE) separable from IgA. Zone electrophoresis was performed for a ragweed-sensitive patient's serum. The serum sample was then mixed with IgA containing isoagglutinin. The solid black line represents the protein concentration. The broken blue line and dot-and-dashed red lines represent hemagglutinin (H-A) and Prausnitz-Küstner (P-K) titers, respectively.⁴

protein was sufficient for sensitizing normal skin. In the second report⁴ they concluded that reaginic antibody is not associated with IgD, IgG, IgM, and IgA by using antibodies against these immunoglobulins. They also found that the molecular size of an unidentified carrier protein of the reaginic antibody was larger than that of IgA having isohemagglutinin activity by using zone electrophoresis and sucrose density gradient ultracentrifugation (Fig 2).⁴ In the third report⁵ they further fractionated the crude IgA fraction containing reaginic activity obtained from patients with hay fever and tested whether the reaginic activity might be present in this further purified fraction. Then anti-IgA was added to the further fractionated preparation to precipitate all IgA molecules, and the Ishizakas found that all reaginic activity in the further fractionated preparation remained in the supernatant. Thus in these 3 articles they concluded that the activity was associated with an impurity often contained in the crude serum IgA fraction, which was not detectable by using the usual immunochemical methods at that time and that the concentration of the carrier protein of the reaginic activity in the original serum was less than 1 $\mu\text{g/mL}$.

In the 1960s, requirements for identification of a new protein were to isolate the protein and to prove that the physicochemical properties of the pure protein are different from those of the known proteins. However, such approaches were impossible for identification of a minor component of the serum. Thus the Ishizakas changed their strategy to prepare rabbit antibodies against this impure fraction showing reaginic activity, in which known immunoglobulin classes were removed. Finally, they were able to identify IgE as a unique protein using newly prepared anti-human IgE.⁷ As all of us know, the discovery of IgE was completed by the discovery of a patient with IgE myeloma by Johansson.⁸

The triumph of the Ishizakas' discovery of IgE went to the 2 articles published in the *Journal of Immunology*.^{1,2} However, it would not have been possible to identify IgE without their preceding studies described in the 3 articles, which were published in the *Journal of Allergy*.³⁻⁵ Studies on "physicochemical properties of reaginic antibody" seem to have been seamlessly performed in a logical fashion.

It is with pride that our *Journal of Allergy* was selected for the publication of the first 3 parts of this series. This shows that even more than a half century ago, our journal had a high reputation in the international scientific allergy community. Today, we are building on this scientific heritage and continue developing the field of IgE in basic and clinical science with great effect on diagnostic and therapeutic strategies. We are still not at the end of this road because our understanding of IgE and beyond is still advancing.

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