The ALLERGY ARCHIVES

Pioneers and Milestones

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The Ishizakas and the search for reaginic antibodies

The author had the good fortune of being a Fellow in the Allergy and Immunology Program at the University of Colorado while the Ishizakas were in Denver. Kimi and Terry would come to the division meetings and present periodic updates on the work in progress. It was clear very early on that they were onto something of major importance. It was a privilege to watch the discovery of a new immunoglobulin as a work in progress.

Kimishige Ishizaka was born in Tokyo, Japan, on December 3, 1925. He received his MD from the University of Tokyo in 1948 and his DrMedSci in Immunology from the same university in 1954. In his presidential address to the American Association of Immunologists in 1985,[1] he credited his interest in immunology to his mentor as a medical student, Professor Keizo Nakamura, who was studying mediators of anaphylaxis at the time.

Teruko Ishizaka was born on September 28, 1926. She received her MD from Tokyo Women's Medical College in 1949 and her PhD from the University of Tokyo in 1955.

Kimi and Terry Ishizaka (Fig 1) were married in 1949. They spent the next 8 years in Professor Nakamura's laboratory studying mechanisms of anaphylaxis. These studies might have influenced their later decision to attempt to identify the skin-sensitizing antibodies (reagins). Concurrently, Kimi Ishizaka also held the titles of Professor of Experimental Research and Chief of the Division of Immunoserology at the National Institutes of Health in Tokyo.

In 1957, Kimi and Terry became postdoctoral fellows in Dan Campbell's laboratory at Caltech. Their research dealt with the biologic activities of soluble immune complexes. An important product of this research was recognition that the ratio of antigen to antibody in the complexes determined some of their biologic activities. For example, complexes that contained 2 or more molecules of antibody would activate complement and elicit inflammatory reactions in skin, whereas complexes that contained 2 molecules of antigen and only 1 molecule of antibody would not. This model of bridging of antibody molecules by

FIG 1. Drs Kimishige and Teruko Ishizaka.

antigens is an important concept in immunology. After 2 years at Caltech and a short time in Manfred Mayer's laboratory at Johns Hopkins, Kimi and Terry returned to Japan and the National Institutes of Health and continued their studies of soluble immune complexes.

The next major career event came in 1962, when Kimishige and Teruko were recruited to the Children's Asthma Research Institute and Hospital (CARIH) in Denver by Dr Sam Bukantz, who was the Medical Director. CARIH was founded in 1907 as the Denver Sheltering Home to provide lodging for the children of patients who were in Denver for the treatment of tuberculosis. It became known as the Home. By the end of the 1930s, fewer patients were coming to Denver for treatment, and the mission of the Home changed to care of children with severe asthma. The name of the institution was changed to the Children's Asthma Research Institute and Hospital. Traditionally, CARIH had been oriented to clinical care of chronically ill children, and development of a basic science research program was a significant new activity for a relatively small and modestly funded institution.

WHICH IMMUNOGLOBULIN HAD SKIN-SENSITIZING ACTIVITY?

It had been shown by Prausnitz and Küstner in 1921[2] that serum from allergic patients, when injected intradermally into nonallergic recipients, would confer local reactivity to the antigens to which the serum donor was allergic. The state of local reactivity lasted for several weeks. Forty years later, the identity of this skin-sensitizing factor was still unclear.

By the early 1960s, 3 immunoglobulin isotypes were known: 7S-gammaglobulin (IgG), 19S-gammaglobulin (IgM), and β2A or γ1A globulin (IgA). IgD was identified shortly thereafter. There was good physicochemical evidence that the skin-sensitizing

antibody was not 7S or 19S gammaglobulin.[3] Electrophoresis of allergic serum indicated that the reaginic activity migrated with the β2 globulins, such as IgA. Similar conclusions were reached when sera were fractionated by means of ion-exchange chromatography or gel filtration; reaginic activity was found in the same fractions that contained the β2A or γ1A globulins. Fireman et al[4] reported that removal of the 7S-gammaglobulins with specific antisera did not remove ragweed specific reaginic activity, but treatment of the same sera with anti-γ1A removed the skin-sensitizing activity. Indeed, 2 reports from the Ishizaka laboratory provided additional evidence for reaginic activity in the β2A globulins. Sera from donors with ragweed allergy were mixed with purified γ2 globulin, β2A globulin, or β2M globulin and then injected into the skin of nonallergic recipients.[5] When the skin sites were challenged with ragweed extract 24 to 48 hours later, the sites that contained the mixture of allergic sera and β2A produced smaller flare reactions than the positive controls, and wheals did not develop. The mixtures containing β2M or the largest dose of γ2 globulin did not show any reduction of the wheal-and flare responses. Thus the β2A appeared to inhibit the Prausnitz-Küstner reaction. Lesser doses of γ2 globulin also reduced the size of the flare reaction and did not produce wheals. The report also showed that inhibition of the reaction by β2A only occurred when β2A was injected before or at the same time as the allergic serum. The second article[6] presented evidence that the inhibitory activity of the β2A globulin was associated with the ''H'' or heavy chain of the protein.

Thus in the early 1960s, the strongest body of evidence favored IgA as the immunoglobulin with reaginic activity.

THE DISCOVERY OF IgE

There were isolated observations that questioned the belief that all reaginic activity was IgA. There were reports of patients who lacked detectable IgA in their sera but who had allergic diseases and positive skin prick test responses. When the Ishizakas attempted to study reaginic activity of anti-blood group substances in the IgA of human serum, no skin-sensitizing activity was found, even though similar studies with sera from ragweed-sensitive patients showed reaginic activity. They correctly hypothesized that the skin-sensitizing activity of allergic sera was a contaminant that was present in the IgA preparations in low concentrations. Their approach to isolation of the skin-sensitizing antibody was to immunize rabbits and guinea pigs with human sera that contained high titers of skin-sensitizing activity. The animal sera were than absorbed with purified immune globulins or myeloma proteins to remove antibodies against IgM, IgG, IgA, and IgD. The goal of this subtractive process was to leave behind antibodies that reacted with an immune globulin other than those that were known. When serum from a ragweed-sensitive patient was analyzed

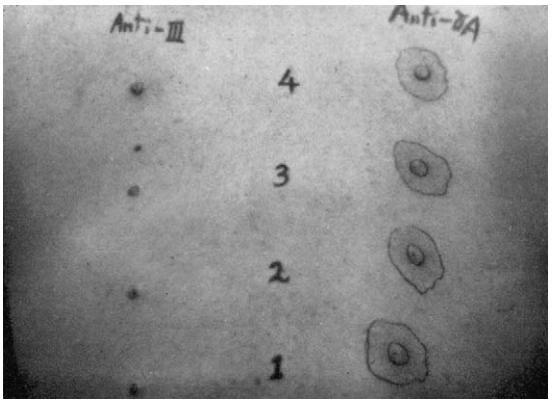

FIG 2. Reaginic antibody activity is not associated with the IgA in atopic sera. Rabbits were immunized with fractions of serum from atopic donors. Antibody activity against IgG and IgA was removed by means of absorption with purified immunoglobulins. By means of immunoelectrophoresis, the remaining antibody (anti-III) identified a protein in atopic serum that was not one of the known serum immunoglobulins. As shown on the *left*, absorption of atopic serum with anti-III removed the skin-sensitizing activity from the serum. Absorption of the same serum with an antibody that was specific for IgA (anti-γA) did not affect skin-sensitizing activity *(right)*. Reprinted from *The Journal of Immunology*, Volume 97, Ishizaka K, Ishizaka T, Hornbrook MM, "Physico-chemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity," Pages 75-85, Copyright 1966, with permission from Elsevier.

by means of immunoelectrophoresis and the gel was developed with the absorbed antisera, a faint band was identified in the γ1 region.[7] When the electrophoretogram was developed with radioactive ragweed antigen E, the radioactivity was localized to the same band. Control experiments showed that the antisera did not react with any of the known serum immunoglobulins. The antibody (anti-III) that precipitated the ragweed antigen-binding protein in serum from atopic donors was then assayed for its effect on Prausnitz-Küstner tests in the skin of nonatopic recipients. Absorption of the atopic sera with anti-III removed the skin sensitizing activity from the atopic sera (Fig 2). In the same experiment, treatment of the atopic serum with anti-γA did not affect the sensitizing activity of the atopic serum (Fig 2). The Ishizakas then proposed that reaginic activity was the property of a previously unrecognized class of immunoglobulins and proposed the name γE.

Concurrent studies showed that γE had many properties in common with other immunoglobulins. The molecules were composed of light chains and heavy chains, and the heavy chains had antigenic determinants that were not shared with other immunoglobulin isotypes. The reaginic property of the molecules was lost when the proteins were reduced and alkylated or heated at 56°C for 4 hours. In 1967, the first IgE myeloma was recognized, and the myeloma protein from this patient shared determinants and properties with IgE from allergic patients. IgE was officially recognized as a new immunoglobulin by the World Health Organization in 1968.[8]

During the late 1960s, many of the biochemical and physiologic properties of IgE were defined.[9] IgE was characterized as a γ2 glycoprotein with a molecular weight of approximately 190,000 d. Class-specific antigenic properties of the Fc portions of the heavy chains were identified. Studies with human sera disclosed that IgE was present in normal sera in minute amounts (100-400 ng/mL) and that greater serum concentrations were common in patients with atopic diseases, parasitic infections, and the hyper-IgE syndrome. Molecular studies disclosed that IgE was divalent and did not fix complement. IgE from allergic sera would sensitize monkey lung tissues so that subsequent challenges with ragweed antigen caused release of mediators, such as histamine and SRS-A. It was shown that IgE bound to mast cells and basophils.

Perhaps the most significant observation of all was the formal demonstration of reaginic activity in IgE of human sera.[10] Sera from subjects with ragweed allergy were processed by using repeated chromatographic steps. The fractions that contained reaginic activity were monitored by means of passive sensitization of human recipients. Ultimately, the regain-containing preparation was shown to bind radioactive ragweed antigen E, but none of the antisera against IgG, IgA, IgM, or IgD bound the antigen. Absorption of the reagin-containing fraction with anti-IgE removed the skin sensitizing activity. An IgA-containing preparation that also had reaginic activity was also studied. Removal of the IgA with anti-IgA did not alter the reaginic activity, but absorption with anti-IgE removed it.

By 1970, there were concerns about the financial health and the future of CARIH. There were rumors of a proposed merger with the National Jewish Hospital and Research Center, also in Denver. It was time to move on, and Kimishige and Teruko Ishizaka moved to the Allergy and Immunology Center at Johns Hopkins.

HONORS AND AWARDS

The work of the Ishizakas did not go unnoticed. They were awarded the Passano Foundation Award, the Paul Ehrlich and Ludwig-Darmstaedter Prize, the Gairdner Foundation International Award, the Borden Award of the American Association of Medical Colleges, the Achievement Award of the American College of Physicians, and the Emperor's Award and Honorary Fellowships in the American Academy of Allergy, Asthma and Immunology. Kimishige Ishizaka was elected to the National Academy of Sciences as a Foreign Associate.

CLOSING COMMENTS

From time to time, it is asked whose skin was used for all of the passive transfer studies. The best guess is that the skin belonged to the investigators themselves. It is also noteworthy that Ms Evelyn Lee, who was a laboratory assistant with the Ishizakas at CARIH, went on to further her education and serve as superintendent of the Denver Public School System.

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The story of IgND

University studies are not a way to support a young family, at least not in Sweden! Thus as soon as I was considered suitably qualified, albeit as a laboratory technician, I jumped at the opportunity to start work. In the fall of 1960, I joined the budding Section of the Blood Transfusion Service at the University Hospital in Uppsala. But routine laboratory work was not really exciting enough, and therefore when my boss, Claes F. Högman, MD, and his colleague Johan Killander, MD, started a research group in the highly topical field of immunochemistry-immunology, I was pleased to be invited to join. My first task was to establish a technique for quantitation of immunoglobulins. From the blood serology aspect, it was of interest to be able to characterize anti-Rh antibodies into the two, at that