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

time, well-known immunoglobulin classes, then referred to as γG and γM, but in addition, we wanted to know more about the structure and function of the immunoglobulins.

For the biochemical studies, another medical student, Hans Bennich, was recruited. Hans was then working on a research project on the separation of serum proteins at the Institute of Biochemistry under the famous Professor Arne Tiselius, one of the Nobel Laureates in this field at Uppsala University.

Hans and I started to work with the immunoglobulins project around 1962, a close collaboration that would continue for more than 20 years. It soon became clear that Hans, in his work to isolate an immunoglobulin, needed sera with high concentrations. It was known that in a rather uncommon disease, multiple myeloma, affecting the immunoglobulin-producing plasma cells, a very high and homogenous increase in the cell product could be secreted. In electrophoresis this product was seen as a narrow and usually strongly stained band referred to as an M-component. I set up immunoelectrophoresis and started to identify each M-component detected in the routine screening of sera using electrophoresis.

The M-components, being either IgG, IgM, or IgA, were purified, and using reduction-alkylation and digestion with proteolytic enzymes, such as papain and pepsin, Hans prepared many fragments of varying size and carbohydrate content, fragments like Fab (fragment antigen binding), Fc (fragment crystallizing; this was actually true only for some IgGs), or light and heavy chains. To be able to characterize the immunologic properties of these fragments, I developed immunoassays using antisera that we raised in rabbits.

Both my clinical immunology activities and Hans' biochemistry work developed nicely, and by 1964, we had developed considerable technical skills and had accumulated large amounts of information on the complex structures of immunoglobulins. We were less good at producing publications, although I did report on our experiences of immunoglobulin quantitation at the Annual Meeting of the Swedish Physicians in 1963. However, some news about our activities must have spread through the grapevine because in January of 1966, a young scientist, Malcolm ''Mac'' Turner, came from Birmingham (UK) to work with Hans. Mac was a nice person and also a very useful link to immunochemistry science in the UK.

In early 1965, I found a serum with an M-component that I could not identify as either IgG, IgM, or IgA. This was very exciting, especially in the light of information claiming that a D. S. Rowe had, during a stay at Johns Hopkins in Baltimore, found an M-component that fulfilled all the criteria for representing a new class of immunoglobulins. Following the alphabetical order, this had been termed IgD. I quickly managed to get in touch with Hans, who was then out of town for a few weeks. He also became excited, and over the phone, he instructed his laboratory technician to start, as a first step, a simple gel filtration, separating serum proteins by size. However, the unusual protein peak had the same position as IgG, and when I analyzed the fractions, I could indeed confirm that the M-component was IgG, which, for unknown reasons, was unwilling to react in serum. This experience was a bit disappointing!

In June 1965, I found a second M-component that I could not identify (Fig 1). This time, to spare Hans the work and me the shame, I did the gel filtration separation myself at my hospital laboratory and analyzed the fractions for IgG, IgM, and IgA. This serum was different; the main protein peak indicated a molecular size similar to that of IgA, but in no fraction could I find the M-component. Could it be one of the subclasses of IgA, which was discussed among immunologists at that time? Could it be an IgA with hidden class-specific antigenic determinants? Maybe it was an IgD? Through Mac Turner, we contacted David Rowe, now back in Birmingham, UK, to get some anti-IgD. Or could it, we wondered, like David Rowe's M-component, be a hitherto unknown product of the uncontrolled growing plasma cells of a patient with myeloma?

With the experiences of the odd IgG some months earlier fresh in our memory, Hans at first was not too excited about my new finding. However, after some weeks, I managed, despite the almost holy Swedish summer vacations, to convince him that this case was even more interesting than the previous one. Thus Hans started to look at the serum and came back excited, both with interesting information and lots of fractions for me to test for immunoglobulin content. Biochemically, the M-component differed from the other immunoglobulins and was referred to as IgX in our laboratory, adopting the Swedish custom to use ''X'' to designate an unknown. A 3.5S fraction, obtained after papain digestion, presumably an FcX, was used to immunize rabbits. It should carry any isotype antigen, and with an anti-IgX, we should be able to find a normal counterpart to the IgX M-component. We had a lot of experience of digesting IgG, and we knew the typical physicochemical properties of the 3.5S Fcγ fragment very well. IgX must be similar, or at least that was what we thought.

The anti-FcX gave a very nice, heavy precipitate with the IgX M-component in gel diffusion and was used in a number of gel-diffusion and hemagglutination techniques to test sera from healthy blood donors and sera from patients sent to me for quantitation of IgG, IgM, and IgA as part of the clinical workup. However, in no case did we see the faintest precipitin line. The idea then came up that the production of IgX could be genetically restricted, and therefore I asked the patient if we could get blood samples from his family members. He was most cooperative and brought his brothers and a highly pregnant daughter.

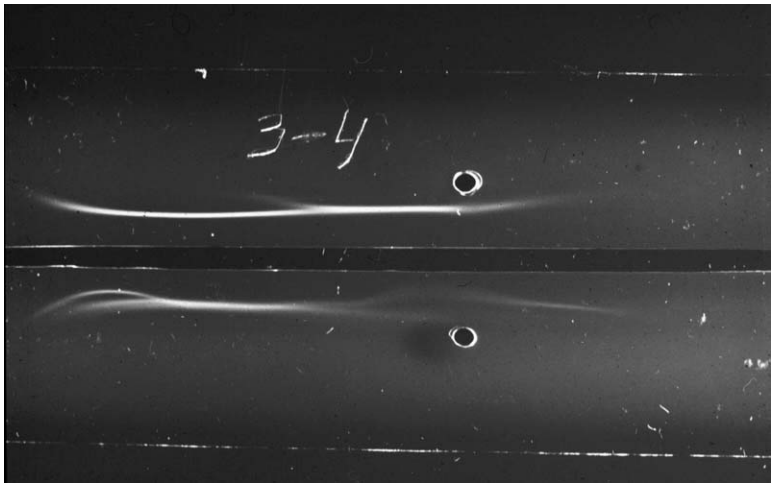

FIG 1. Immunoelectrophoresis of a normal serum *(top)* and myeloma serum ND *(bottom)* by using an antiserum pool *(center lane)* containing antibodies to IgG, IgA, IgM, and κ and λ light chains. Note the faint line of the M-component despite the high protein concentration. The thin line to the left represents free light chains in the myeloma serum.

When their sera were tested, a strong beautiful precipitin line was obtained with the sample from the daughter. Great! Everything was crystal clear: IgX was indeed a new immunoglobulin class, and it was linked to pregnancy. The discovery was celebrated.

There was just one small annoying fact: the precipitate was very strong and seemed to fuse with the positive control serum, which was the purified IgX M-component. Maybe we should repeat the test, but should we use a fresh serum sample? In the meantime the daughter had given birth to a healthy child, and she was still on the ward. Thus I went to see her. Sitting at her bedside, probably looking very ''nondoctorish,'' I convinced her to give us a new sample. Some 30 years later, she confided that my visit had scared her! But that sample was negative. Maybe the nonserious advice to ''celebrate every successful experiment'' should be more generally adhered to.

There we were, stuck in a corner with an immunoglobulin with a unique structure and unique antigenic properties but without a normal counterpart.[1] So back to basics! The papain digestion was repeated, but all 5 or so major fractions with sizes from 3.5S to 7S were used for immunization. Leif Wide, assistant professor at the Department of Clinical Chemistry, was consulted about the possibility of adapting his very sensitive techniques for human chorionic gonadotropin and other hormones already used by us for IgG and IgM to measure IgX.

These projects took most of 1966. During that time, we obtained several anti-IgD antisera, none of which reacted with the IgX M-component.

At the Swedish Physicians Meeting in November 1966, I reported on the unique IgX M-component. There was no real excitement. One world-famous Swedish clinical chemist told us that odd M-components were common, he in fact had several, collected over some years, in his freezer, and they were nothing to be excited about. Interestingly, later, using anti-IgD from David Rowe, he found that 4 of these were IgD myelomas.

We had now worked with the IgX M-component for 18 months, and we still had not found any counterpart in normal serum. A month later, in January 1967, everything changed. The radioimmunoassay used (Fig 2) on the basis of the antiserum to the 7S fragment of IgX was inhibited in a dose-response way by sera of blood donors. We suddenly had all the evidence needed for definition of a new immunoglobulin class: a unique biochemical structure carrying unique antigens generally present in the population. According to the convention at that time, it was provisionally named using the initials of the patient from whom it originated, IgND. Actually, a couple of years ago, I heard a plenary speaker at an international congress tell the audience that ''ND'' was the abbreviation of ''not detected''!

Among the very first blood donor sera tested for IgND to represent a reference population was a remarkable outlier with an IgND level about 30 times the mean of the group. In all the studies I had performed on serum levels of IgG, IgM, and IgA, neither in normal nor in pathologic sera had I ever seen such an outlier. I contacted the donor, a young girl.

She insisted on being healthy but did admit that she had experienced some breathing problems a couple of weeks earlier, when she had spent her Christmas holiday with her parents. They had a dog. I asked a friend at the outpatient allergy clinic to have a look at her, and he confirmed dog allergy–induced asthma.

In my freezers I had many sera from patients with asthma. I had been quantitating serum IgG levels for a clinician who treated asthma with gammaglobulin injections. I quantitated IgND in about 50 of these sera and asked him, without knowledge of my results, to

FIG 2. I am working in the laboratory washing particles in the centrifuge, while Hans, to the right, is watching. This is not a picture of real laboratory work but a show for a local newspaper, probably in 1967.

classify the patients as being allergic or not allergic. Serum IgND was increased in about 60% of the sera from patients with allergic asthma.[2]

We were a bit concerned about these findings. As we understood it, it was definitely proved that the serum activity capable of mediating an allergic reaction, referred to as a ''reagin,'' was linked to IgA.[3] Ishizaka et al[4] had provided the final evidence by showing that isolated heavy chains of IgA could block the Prausnitz-Küstner (PK) test and thus did have the structure critical for a reagin molecule.

Because IgND seemed to be linked to allergy, we, together with Leif Wide, developed a radioimmunoassay for IgND antibodies to common allergens. The antiglobulin principle, used daily at the blood center for tracing anti-Rh antibodies in the sera of pregnant women, was adopted. The proteins of commercial allergen extracts were covalently coupled to cyanogen bromide–activated cellulose particles. The test, called the RAST, gave results that correlated well with the clinical allergy diagnosis.[5] However, in this work I had the first encounter with supposedly standardized allergen extracts, and it was rather shocking; they varied 100- to 10,000-fold in allergen content measured as IgND-binding capacity.

The study of IgND in asthma, the report on the RAST, and findings of a dose-response blocking of the PK test with purified IgND, performed by Dr Denis R. Stanworth in Birmingham,[6] were all published in *Lancet* in the fall of 1967. However, in March 1967, we had contacted the Ishizaka group and informed them about IgND and invited them to compare reagents. In recent articles they had reported that they had an antiserum to something they called γE. It was said to be able to block the factor causing erythema to ragweed and, more interestingly, the reagin activity to ragweed allergen E in PK testing. The Ishizakas were excited and sent us some anti-γE, and we sent them an anti-IgND and some purified IgND. This was the start of several years of scientific collaboration and personal friendship.

Together we approached the World Health Organization International Immunoglobulin Reference Centre. Both groups sent them what we had available of antisera, and Hans and I also sent purified IgND. Unfortunately, because of the very low concentration in serum, it had not been possible to purify γE. In February 1968, Hans, Kimi, and I, together with a couple of collaborators and experts, spent a week at the World Health Organization Centre in Lausanne. The conclusion of this workshop was that the unique biochemical and antigenic properties of IgND, supported by the unique antigenic properties of γE, represented a new immunoglobulin. The very first report was written,[7] announcing the presence of the new immunoglobulin class, IgE, taking the fifth and, as it turned out, final alphabetical position.

The discovery of IgE has had a very positive effect on the clinical and scientific aspects of allergy, and the local and international collaborations that resulted in its formal recognition are in the greatest scientific tradition. It has also had a very positive personal effect, and I have been greatly honored—for example, some 20 international allergy societies, including the American Academy of Allergy, Asthma and Immunology and the American College of Allergy, Asthma and Immunology, have kindly nominated me as an Honorary Fellow.

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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-γE-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 (γG) and IgM (γM). Many of the key scientific observations leading to this conclusion are listed in Table I. After identification of IgA (γA, originally known initially as β2a globulin) in 1959, many researchers began examining the potential association between human IgA and reaginic activity in serum. The γA fraction of human atopic sera was initially shown to have skin-sensitizing activity, and γA globulin but not γG or γM 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 μg nitrogen/mL antibody was required for a positive reaction. However, because some reaginic sera with a γA concentration of 0.3 mg nitrogen/mL titered out to 1:10,000, this would have meant that all of the γA in these sera would have had be specific for a single allergen specificity (which was considered unlikely). To shed further doubt on the association between reaginic activity and γA, a serum was identified with reaginic activity that lacked any detectable γA. Finally, human anti-γA isoagglutinin studies indicated no detectable γG, γA, or γM 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 γA or γG levels. Precipitation of γG and γA from these fractions with γG and γA 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