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The Way We Walked with Immunology

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Abstract

It has been a little more than 50 years since we discovered IgE, a key molecule for the allergic response and a target for treating allergies and severe asthma. Here, I trace my career, from the kindling of my interest in immunochemistry to groundbreaking discoveries in the biology and chemistry of immunoglobulins. I describe my service to the broader community of immunologists and my role in shaping departments and research institutes. My course starts in Japan and includes Southern California, Baltimore, and Denver.

OUR INTRODUCTION TO IMMUNOLOGY

When I was accepted to the medical school of the University of Tokyo in 1944, I was expecting to become a practicing physician. However, after I spent my summer vacation in 1946 training in bacteriology with Professor Keizo Nakamura at the Institute of Infectious Diseases, I became interested in basic science. At the end of this training, Professor Nakamura asked me to translate a book entitled *The Chemistry of Antigens and Antibodies*, written by Professor J. R. Marrack in 1938. It took three months for me to translate the volume into Japanese. I was very much impressed by the contents of the book, and I then became interested in immunochemistry.

In 1947, by the order of the United States Army, the Institute of Infectious Diseases was split to establish a separate National Institute of Health (NIH) in Japan, and Professor Nakamura was transferred to this new organization. Nevertheless, I spent summer vacation every year in his laboratory to do some experiments, and I made up my mind to focus on immunology after my graduation from medical school.

My wife, Teruko (Terry) Ishizaka, had similar experiences. When she was a medical student, she spent two weeks in the winter of 1946 in Professor Nakamura's laboratory to learn bacteriology. In April 1947, Professor Nakamura invited us to his home, and I was introduced to Terry. After I completed training in clinical medicine and earned a medical license, we married in December 1949.

POSTDOCTORAL TRAINING BY PROFESSOR DAN CAMPBELL

I received a PhD from the University of Tokyo in 1954 and then worked in Professor Nakamura's laboratory at the NIH, Japan. Professor Nakamura wanted me to receive postdoctoral training from Professor Dan H. Campbell at the California Institute of Technology (Caltech), in Pasadena, California, USA. As a result of Professor Nakamura's recommendation, I had the chance to work in Professor Campbell's laboratory, beginning September 1, 1957.

After I received general training for two weeks at Caltech, I asked Professor Campbell to suggest a research project for me. At that time, he anticipated that soluble antigen-antibody complexes might have biologic activities, which are lacking in either the antigen or antibody alone, and that the formation of bioactive antigen-antibody complexes in vivo might be responsible for the induction of anaphylaxis. He talked about his idea and asked me to test his hypothesis. When I asked him about the appropriate experimental system to be used, he stated, "That is your business! You may decide on an appropriate experimental system for testing my hypothesis" (**Figure 1**).

As rabbit antibodies can sensitize the skin of normal guinea pigs for passive cutaneous anaphylaxis, I wondered if the soluble complexes of antigen with rabbit antibody, such as bovine serum albumin (BSA)–anti-BSA antibody complexes, could induce an increase in the permeability of skin capillaries in normal guinea pigs. However, the γ-globulin fraction of rabbit antiserum, obtained by salt precipitation, induced an increase in the permeability of normal guinea pig skin capillaries by itself. Fortunately, the skin-reactive substance in this fraction could be separated from the anti-BSA antibodies by starch block electrophoresis. The slow-moving γ-globulin fraction of the antiserum contained approximately 50% of the antibodies in the antiserum, but it did not contain the skin-reactive substance. When I obtained these results, Professor Campbell came into our laboratory, looked at the data, and said, "Oh! This is useful." The next morning, he called me to his office and mentioned, "We have a so-called Federation meeting in April, and postdoctoral fellows have a chance to present their papers before the meeting. You may present your paper." As I did not yet have any data on the biologic activities of the soluble antigen-antibody complexes, I asked him when would be the deadline for submission of the abstract. He answered, "It is about Christmas time," and I then asked, "Do you think I can get enough data to prepare the abstract within three months?" He answered, "Why not? You can do it!"

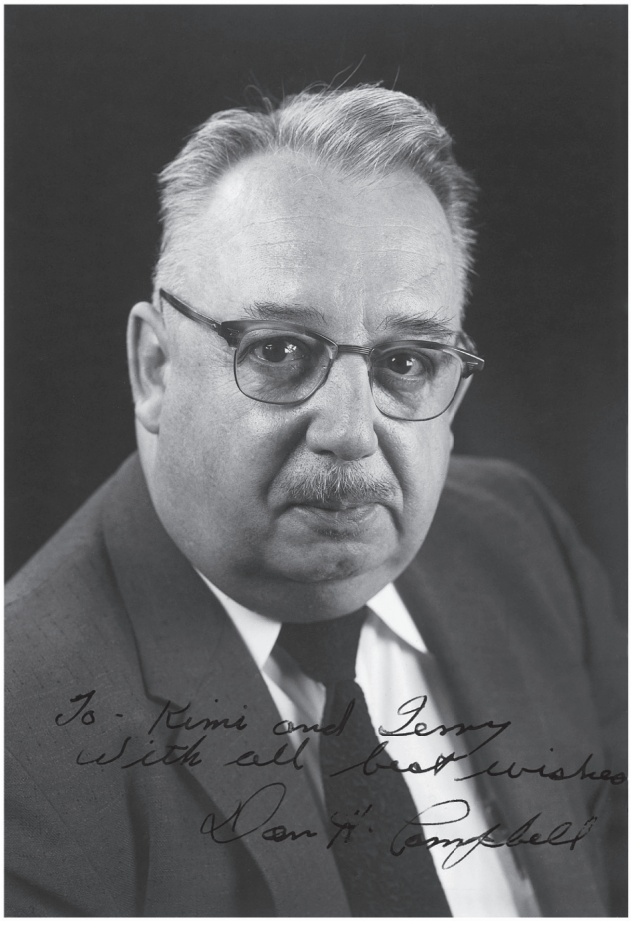

Figure 1

Professor Dan H. Campbell (1908–1974). The keynote lecture at the Midwinter Immunology (Asilomar) meeting is named in his honor.

Fortunately, our experiments revealed that the soluble BSA–anti-BSA complexes obtained with slight antigen excess induced skin reactions in normal guinea pigs; however, complexes formed with extreme antigen excess failed to do so. Analysis of the soluble ^{35}S-labeled BSA–anti-BSA complexes by free-boundary electrophoresis revealed that the complex composed of one antibody molecule and two antigen molecules (Ag_2Ab) failed to induce the skin reaction, whereas the Ag_3Ab_2 complex, and those complexes poorer in BSA antigen content, had the ability to induce an increased permeability of skin capillaries of normal guinea pigs. The data indicated that the binding of two antibody molecules to a common antigen molecule is required for the formation of the skin-reactive antigen-antibody complex (1), suggesting that cross-linking of two antibody molecules by multivalent antigen is responsible for the induction of anaphylactic reactions (**Figure 2**).

I wondered whether the same principle might apply for other antigen-antibody systems. To test this possibility, I asked Professor Campbell to get various antigens and antisera. He brought me down to the animal quarters, where he had a Revco freezer full of 50-mL bottles containing

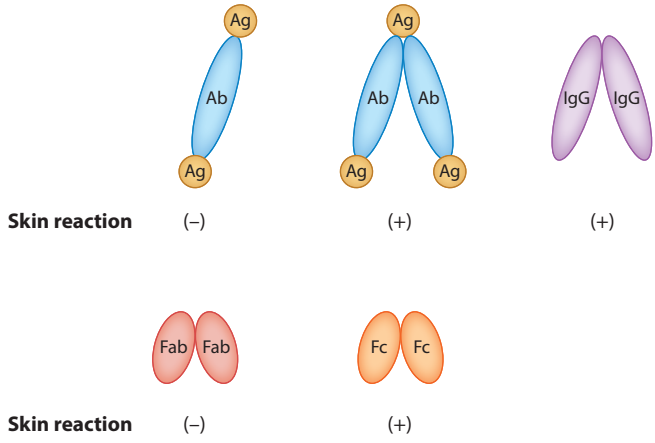

Figure 2

Scheme of the cross-linking required to obtain anaphylaxis. Aggregated IgG also has activity (*upper right*), as does the aggregated Fc portion (*lower right*).

antisera against various antigens or purified antigens. When I came back to our lab, with various antigens and antisera, Professor Campbell came again and mentioned, "Prepare your manuscript before you begin your experiments. I am not kidding you. Karl Landsteiner always prepared his manuscript before he began experiments. You can do it!"

After I prepared my manuscript based on my speculation, Professor Campbell allowed me to begin experiments. The results of the experiments showed that the biologic activities of soluble antigen-antibody complexes depended on the animal species from which the antibodies are derived, but not on the chemical nature of the antigen involved (2).

As we did not know the possible role of complement in guinea pig tissues for the induction of the skin reaction by soluble antigen-rabbit antibody complexes, I went to see Professor Manfred Mayer, who was an authority on complement. Professor Mayer worked at the Johns Hopkins Medical School in Baltimore, Maryland; therefore, I could visit on my way to Philadelphia, Pennsylvania, where the Federation meeting was being held. When I was speaking with Professor Mayer, Dr. Abraham Osler came in and told me, "I have seen your paper. You should move to Johns Hopkins to study the possible role of complement for the induction of anaphylactic reactions by soluble antigen-antibody complexes." As our work at Caltech was progressing, I did not want to move to another institution. During the Federation meeting, however, Dr. Osler negotiated with Professor Campbell for the opportunity to recruit us to Hopkins, and he told me, "As Dr. Campbell agreed with me, you should move to Johns Hopkins next year." Although I wanted to stay at Caltech, Professor Campbell mentioned, "As you came from Japan, it may be worthwhile for you to spend some time on the East Coast, which is very different from the West." Thus, we had to move to Johns Hopkins Medical School in January 1959.

At Johns Hopkins, every morning Dr. Osler gave me the experimental protocol he had designed. After I completed the experiments and gave him the results, I was allowed to spend some time to continue our work on soluble antigen-antibody complexes. However, Dr. Osler did not have a sufficient amount of rabbit antiserum for the preparation of soluble antigen-antibody complexes. Thus, I asked him to purchase human γ-globulin (HGG) for clinical use, isolated monomeric γ-globulin, and heated the purified HGG solution at 63°C. The polymerized HGG was separated from monomeric HGG by sucrose density gradient ultracentrifugation. As expected, the polymerized HGG induced the increase of permeability of normal guinea pig skin capillaries and

fixed guinea pig complement, whereas monomeric HGG failed to do so. On a weight basis, heat-aggregated HGG was comparable to antigen-antibody complexes, namely, rabbit BSA–anti-BSA complexes, in the ability to induce the skin reactions in normal guinea pigs and to fix complement (3). The skin reactivity and complement-fixing activities also were induced by polymerization of human IgG coupling with bis-diazotized benzidine, as well (4). The results strongly suggested that polymerization of antibody molecules by antigen was responsible for the induction of skin reactivity and complement fixation by the formation of antigen-antibody complexes (**Figure 2**).

At that time, Professor Mayer and his group had already isolated guinea pig complement components, C′1, C′2 and C′4, and had been studying the order of fixation of the C′ components to sheep erythrocytes sensitized with rabbit anti-erythrocyte antibodies. As Professor Mayer was very much interested in the fixation of complement by aggregated normal human IgG, Terry moved to his laboratory to learn complementology, and she determined the order of fixation of C′ components to aggregated HGG: C′l, C′4, C′2—exactly the same order of binding to sensitized erythrocytes (5). The results indicated that complement fixation upon antigen-antibody reactions was the consequence of polymerization of the antibody (γ-globulin) molecules.

From time to time, I spoke with Professor Campbell by telephone and let him know the progress of our research at Hopkins. He advised us to continue the research on the biologic activities of soluble antigen-antibody complexes, even after our anticipated return to Japan. At that time, however, the Japanese government did not have a system for providing medical research grants. Therefore, it was impossible for young scientists to get funds for basic research from the government. Professor Campbell suggested to me, "If money is the problem, you may apply for a research grant from the US NIH. They have a system to support outstanding research in foreign countries." Thus, I visited the US NIH to learn how to apply for a research grant. As Dr. Osler took a long summer vacation in Europe, we moved back to Caltech in the beginning of August 1959, prepared and submitted our research grant application to the NIH, and went back to Japan in September. Because we received our NIH grant, we could continue our research on the biologic activities of soluble antigen-antibody complexes in Japan.

THE REASON WE DID NOT STAY IN JAPAN

After we came back to Japan, I found an article published by Professor Rodney Porter on the papain hydrolysis of rabbit antibody molecules into three fragments (6). As I anticipated that the abilities of antigen-antibody complexes to induce allergic reactions and to fix complement were based on some structures in a portion of the antibody (γ-globulin) molecules, I digested anti-BSA rabbit antibodies by crystalline papain to obtain Fragments I, II, and III of the antibodies. As Professor Porter had described, Fragments I and II (Fab fragments) contained an antigen-binding site, whereas Fragment III (the Fc fragment) did not. Thus, we polymerized each fragment by coupling with bis-diazotized benzidine and tested for the biologic activities of the polymerized fragments. The results clearly showed that the polymerized Fc fragment induced skin reactions in normal guinea pigs and fixed complement, whereas neither the monomeric Fc fragment nor the polymerized Fab fragment induced the skin reaction or complement fixation (**Figure 2**). We also found that the monomeric Fc fragment, injected intradermally into guinea pigs, prevented passive sensitization of the skin site with rabbit antibodies, whereas the Fab fragment failed to do so. The results indicated that the antibody structures involved in passive sensitization of guinea pig skin and those essential for C′ fixation were present in the Fc portion of the antibody molecules (7).

As the most important question regarding antibodies for immunologists was the structural basis for their capacity to bind diverse antigens specifically, our studies on the role of the Fc portion of the antibody and its biologic activities did not gain much attention in Japan. However, I presented

our paper "The Role of the Fc Portion of the Antibody Molecule in the Induction of Anaphylaxis and C′ Fixation" at the Fourth International Congress of Allergology, which was held in New York City in 1961, and visited several institutions to give seminars on my way back to the West Coast.

After discussion with several allergists during the trip, I began to wonder if the principles ascertained by our studies on soluble antigen–rabbit antibody complexes could elucidate the mechanisms of allergic reactions in humans. I anticipated that the best system to test this possibility would be the Prausnitz–Küstner (PK) reaction, first described in 1921 (8). Dr. Karl Prausnitz injected serum from an allergic individual, Dr. Heinz Küstner, into his own arm. The next day he followed this with challenge at the injection site with the allergen to which Küstner was sensitive, and an erythema-wheal reaction resulted. Because antibody specific for the allergen in question was not detectable in the serum of Küstner at that time, the serum substance responsible for induction of the skin reaction was called "reagin." I anticipated that reagin was a type of antibody specific for the allergen and speculated that formation of allergen-reagin complexes in skin tissues would be responsible for the induction of allergic erythema-wheal reactions in humans.

Reagin was required to test the hypothesis. However, hay fever patients were not identified in Japan until 1964. (The first case was a patient who was allergic to cedar pollen.) I therefore considered whether I should continue our research on soluble antigen–rabbit antibody complexes and the Fc portion of the antibody molecules in Japan or instead move to an institution in the United States to investigate possible mechanisms of the PK reaction by using reagin from the serum of hay fever patients.

During my trip to New York in 1961, I received offers from several institutions in the United States. Among them was Scripps Clinic, which had just been established in La Jolla, California, by Dr. Frank Dixon. At that time, his group had been studying the role of antigen-antibody complexes in the induction of glomerulonephritis; and I had several friends and competitors at Scripps Clinic. However, Dr. Sam Bukantz negotiated with Dr. Dixon and tried to recruit us to the Children's Asthma Research Institute and Hospital (CARIH) in Denver, Colorado. CARIH was an unknown, small institute where Dr. Bukantz had been carrying out clinical research on patients with asthma; however, he wanted to recruit basic immunologists to CARIH. As he proposed that CARIH would purchase all the major equipment required for our research and would set up our laboratories, Dr. Dixon advised me to accept the offer from Dr. Bukantz. Thus, I decided to move to CARIH to test the possibility that the principles of the immunological mechanisms of anaphylactic reactions in experimental animals might elucidate the mechanisms of reaginic hypersensitivity in humans.

FROM HELL TO HEAVEN IN CARIH

When we arrived in Denver in October 1962, friends met us at the airport and told us that Dr. Bukantz would leave the institute by the end of June 1963. On the next day, I went to CARIH and found an empty laboratory with no major equipment for us. As our NIH grant to CARIH was already effective, I began to set up our laboratory. Dr. Bukantz had been in charge of both the hospital and the research institute at CARIH, and his three technicians had been employed by means of his research grants. They would lose their jobs after Dr. Bukantz resigned.

As my purpose for moving to CARIH was to study immunological mechanisms of reaginic hypersensitivity, I asked one of the doctors at CARIH to take a small amount of blood from asthma patients and tested it with the PK reaction for the presence of reagin, using my arm as the recipient. It was to my great surprise that even a 1:1,000 dilution of the sera was sufficient for sensitization of my skin for the PK reaction. Since our NIH research grant proposal did not include any plan to study human reagin, I prepared applications for supplemental research grants from the NIH and the National Science Foundation to equip our laboratories to study the nature

of reagin. I also included the salary of two of Dr. Bukantz's technicians in the budget of my grant application to let them participate in our research on reagin.

Beginning in the 1950s, several investigators studied physicochemical properties of reagin in the sera of hay fever patients using various fractionation methods, such as zone electrophoresis, ion exchange column chromatography, and ultracentrifugation. The results clearly showed that the physicochemical properties of reagin were different from the properties of IgG or IgM. After a third immunoglobulin isotype, IgA, was characterized, Heremans & Vaerman (9) reported that apparently pure IgA preparations obtained from the sera of hay fever patients had skin-sensitizing activities, and these results were confirmed by several other investigators. By the end of 1964, most immunologists and allergists believed that reagin was an IgA antibody specific for the allergen. Thus, I decided to study the immunological properties of IgA antibodies. For this purpose, we immunized blood group O individuals with the A substance from blood group A, isolated IgA, IgG, and IgM from their sera, and determined the immunological properties of IgA antibody specific for "substance A" in comparison with those of IgM and IgG antibodies specific for the same antigen (10).

To my great surprise, none of the IgG, IgM, and IgA antibodies specific for the blood group substance sensitized human skin for the PK reaction. As I believed that the biological activities of antibodies were based on the Fc portion of the antibody molecules, I could not accept that the anti-ragweed IgA antibody in the sera of hay fever patients had skin-sensitizing activity and that the IgA antibody against substance A did not. I considered whether the reaginic activity in the IgA fraction of the sera of hay fever patients might be associated with a minor component present in the IgA fraction as an impurity. To test this possibility, I added the rabbit antibodies specific for human IgA to the IgA fraction of the sera of hay fever patients to precipitate all the IgA, and tested the supernatant for the presence of reagin by the PK reaction. The results clearly showed that all reaginic activity in the IgA fraction remained in the supernatant, indicating that reagin in the IgA fraction was associated with an impurity that was not detectable by the usual immunochemical methods (11). However, this finding brought up another problem. Since the reaginic activity (PK titer) of the supernatant was comparable to the activity of the original sera, from which the IgA fraction had been obtained, our results suggested that the concentration of the protein that carried the reaginic activity in the original sera was less than 1 μg/mL.

At that time, identification of a new protein required isolating the protein and then proving that the physicochemical properties of the pure protein were different from those of known proteins. However, such approaches were impossible for the identification of a minor component of the serum. Thus, I switched our strategy and decided to prepare rabbit antibodies specific for reagin and to identify the human serum protein having the reaginic activity in vitro by using the anti-reagin antibodies.

For this purpose, we immunized rabbits repeatedly with the reagin-rich fraction of the sera from hay fever patients, together with complete Freund's adjuvant. The rabbit antiserum was absorbed with normal human IgG, IgA, and IgD myeloma proteins to remove all antibodies reactive to the known human immunoglobulin isotypes.

To test for the presence of antibodies specific for reagin in the absorbed antiserum, the γ-globulin fraction of the antiserum was added to the reagin-rich fraction of the serum of a ragweed-sensitive patient. After incubation for 24 h at 5°C, the mixture was centrifuged to remove a trace amount of precipitates, and the supernatant was tested for the presence of reagin by the PK reaction. As shown in **Figure 3**, reagin was removed by the antiserum, indicating that the rabbit antiserum contained antibodies specific for reagin (12).

Therefore, we set up radio-immunodiffusion and radio-immunoelectrophoresis to identify the reagin in vitro by using the rabbit anti-reagin antibodies and radio-labeled ragweed antigen E. Since the protein forming the precipitin band did not belong to any of the known immunoglobulin

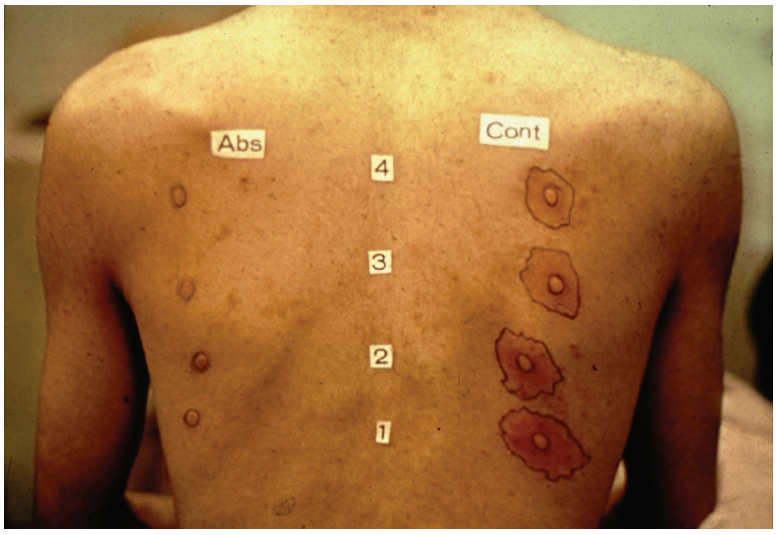

Figure 3

Antiserum to reagin absorbs the activity that causes the PK reaction (antibodies, *left*). The reaction is indicated by the erythema-wheal reaction in the control (*right*).

isotypes but the protein contained the antibodies specific for ragweed antigen E, we tentatively called the protein γE; I believed that the human anti-ragweed antibodies detected by the rabbit antiserum were responsible for induction of erythema-wheal reactions (12).

Similarly, the globulin fraction of the sera of ragweed-sensitive patients was fractionated by DEAE cellulose column chromatography, and the distribution of reagin in the chromatographic fractions was determined by using the rabbit anti-reagin (γE) antibodies. In the agarose plate shown in **Figure 4**, the peripheral wells were filled with the original serum (S) and the chromatographic fractions, and the center well was filled with the anti-γE antiserum, followed by ^{131}I-labeled ragweed antigen E. Autoradiography of the plate indicated that the precipitin band formed by Fraction II contained the highest antibody activity specific for antigen E. Determination of the anti-ragweed reaginic activity of each fraction by PK tests also showed that Fraction II had the highest skin-sensitizing activity (13).

The reagin-rich fraction of the sera of ragweed-sensitive patients was fractionated further by sucrose density gradient ultracentrifugation and gel filtration, and the distribution of γE was determined by radio-immunodiffusion using the anti-γE antibodies and radiolabeled antigen E. The results showed that the sedimentation coefficient of γE was 8.0S and the molecular weight of the protein was approximately 200 kDa, indicating that the physicochemical properties of γE were different from the properties of the known immunoglobulin isotypes. It was also found that γE had both κ and λ light chains, indicating that γE represented a unique immunoglobulin isotype (13).

Definitive evidence that convinced us of the γE character of reagin resulted from measurement by radio-immunoassay of the antigen-binding activities of IgG, IgA, and γE antibodies against purified ragweed antigen E from the sera of ragweed-sensitive patients. The antigen-binding activities of γE antibodies in the sera correlated with the maximal dilution of the sera giving positive PK reactions. No correlation was observed between the PK titer and the antigen-binding activities of IgG or IgA antibodies in the sera, indicating that the intensity of

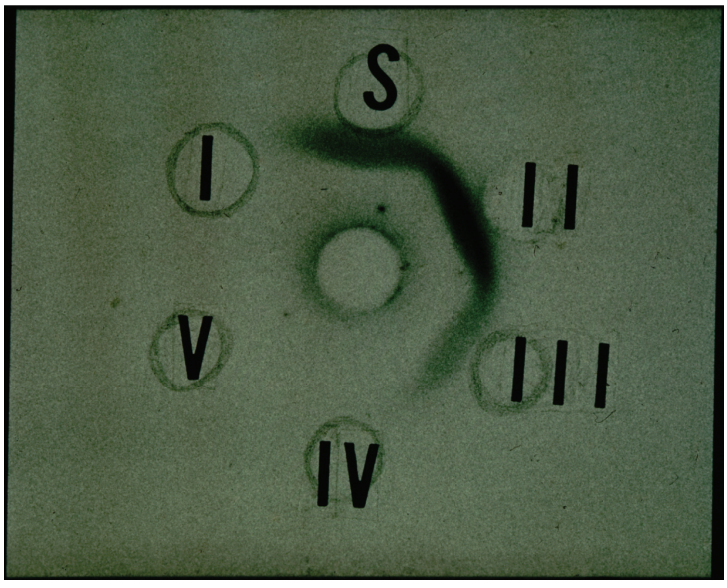

Figure 4

Radio-immunodiffusion to identify reagin. Fraction II of the serum (S) has the highest activity.

the skin-sensitizing activity of a patient's serum, determined by the PK reaction, was due to the concentration of γE antibodies specific for the allergen in the serum (14).

Based on these findings, we isolated γE from the sera of ragweed-sensitive patients by combining several fractionation methods, and a trace amount of IgG and IgA remaining in the γE preparation was removed by using homemade anti-IgG and anti-IgA immunosorbents. The final preparation of γE, in which none of the other immunoglobulin isotypes was detected, gave PK reactions up to the dilution of 1:80,000. Thus, we concluded that reagin is the γE antibody specific for the allergen (15).

The rabbit anti-γE antibodies were also useful for identification of the first two cases of IgE myeloma patients. At that time, Drs. S. G. Johansson and H. Bennich, in Sweden, had isolated an atypical myeloma protein (16), and they recognized that the physicochemical properties of the myeloma protein were almost identical to the properties of γE that we described in our publication (13). Therefore, they contacted us, and we exchanged our rabbit anti-γE antiserum for their sheep antiserum specific for the atypical myeloma protein. As expected, their antiserum and our anti-γE formed a precipitin band of identical specificity with γE, which was isolated from the sera of ragweed-sensitive patients, indicating that the myeloma protein possesses the γE-specific antigenic determinant (17). The unique physicochemical properties of γE, together with the presence of the myeloma protein having the antigenic determinant specific for γE, were sufficient to conclude that γE represents a unique immunoglobulin isotype. Thus, the World Health Organization officially named this protein immunoglobulin E (IgE) in 1968.

Identification of IgE facilitated elucidation of the immunological mechanisms of allergic reactions. In the course of our studies, we found that intracutaneous injection of as little as 1–2 ng/mL of anti-IgE into healthy subjects resulted in an erythema-wheal reaction (18). Because healthy subjects should have IgE, the mechanism for the skin reaction was considered to be the result of binding of anti-IgE to IgE on a normal cell type. It was also found that incubation of normal leukocytes with rabbit antibodies specific for human IgE induced the release of histamine from the

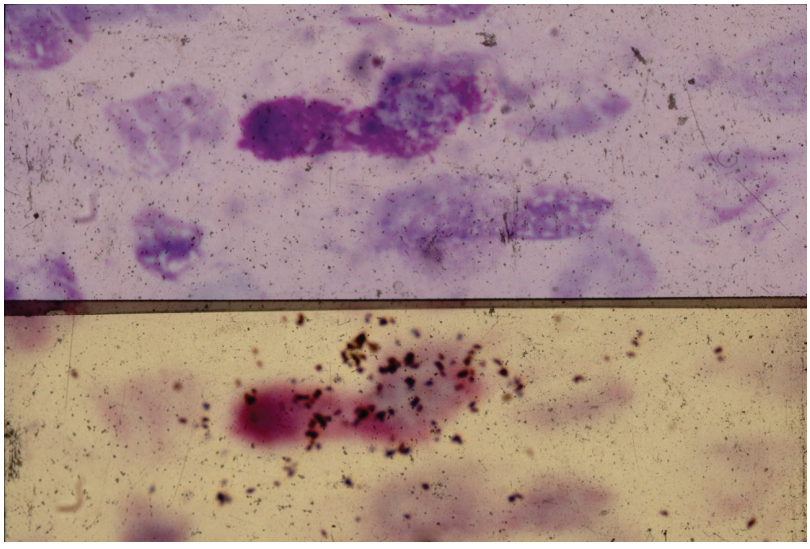

Figure 5

Autoradiograph showing binding of ^{131}I-labeled anti-IgE antibody (*lower*), indicating that cells have receptors for IgE on their cell surface.

leukocytes (19), and that human IgE antibodies sensitized monkey lung tissues and mediated the antigen-induced release of histamine and leukotrienes from the tissues (20). These results strongly suggested that IgE antibodies were involved in pathogenesis of allergic diseases.

Based on this information, we tried to identify the target cell that binds IgE. Since the second IgE myeloma protein became available for our research, an anti-IgE antibody could be specifically purified by using the myeloma protein. The ^{131}I-labeled anti-IgE antibody specifically bound to basophils (21) and mast cells (22), indicating that these cells had receptors for IgE on their cell surface (**Figure 5**). It was also found that the IgE myeloma protein bound to the receptors on these cells through the Fc portion of the molecules (23) and that cross-linking of the cell-bound IgE antibody by a specific antigen was responsible for activation of basophils and mast cells for the release of chemical mediators, which increased permeability of small vessels and caused contraction of smooth muscle (24). The results strongly suggested that IgE antibodies specific for an allergen played an important role in the pathogenesis of atopic diseases.

RESEARCH AND TRAINING OF YOUNG INVESTIGATORS AT JOHNS HOPKINS

In June 1969, Professor Mayer at Johns Hopkins called by telephone and asked me to give a seminar on IgE at the medical school. As I was used to giving lectures on IgE almost every month at many institutions, I accepted his invitation and went to Baltimore. To my great surprise, several famous professors from the Johns Hopkins School of Medicine were sitting in the front row to listen to my talk, and a series of appointments with them had been set up for the next day.

At that time, Johns Hopkins School of Medicine was planning to establish an Immunology and Allergy Research Center, and a five-story research wing was under construction at an affiliated hospital for that purpose. They had already decided to move the Divisions of Allergy, Rheumatology, and Renal Diseases in the Department of Medicine to the research wing and planned to use

Figure 6

Johns Hopkins Director of Medicine Professor A. McGehee Harvey (1911–1998) and Dr. Teruko Ishizaka, in 1980 at Johns Hopkins medical school.

one floor for basic immunologists, who would consult for the immunology research in the three divisions. As we were comfortable in Denver, I did not have any intention to move. However, I was very much impressed by Professor A. McGehee Harvey, Director of the Department of Medicine. He anticipated that immunology would facilitate new clinical research in allergology and rheumatology, and he wanted to have our group at the Immunology and Allergy Research Center.

Some of the major developments in immunology at that time were made in the field of immunochemistry, which did not have much connection to clinical medicine, so I greatly appreciated Professor Harvey's view that the immune system would be involved in the pathogenesis of many diseases. Therefore, after my second visit, I accepted the offer from Hopkins to become a Professor of Medicine and Microbiology there (**Figure 6**).

At Johns Hopkins, I asked Terry to focus her research on the IgE/basophil/mast cell system, and I began to study the mechanisms involved in the IgE antibody response. By that time, mechanisms for antibody responses had been investigated by cellular immunologists, and the collaboration between antigen-specific T cells and B cells for generating antibody responses was a major subject of their interest. As I was an immunochemist, I speculated that the T-B interaction might result in the formation of soluble factors (cytokines) by T cells and that the soluble factors from T cells might play an important role in the differentiation of B cells into antibody-forming cells.

At that time, IgE had been identified in humans, other primates, rats, and rabbits but not yet in mice. Therefore, I decided to study the mechanisms involved in the secondary IgE anti-hapten antibody response using mesenteric lymph node cells from rabbits immunized with hapten-protein conjugates adsorbed to aluminum hydroxide gel. As the rabbit mesenteric lymph nodes are big, the cells from a single rabbit were sufficient for setting up more than 30 sets of cultures. The lymph node cells were treated with the appropriate antigen for 24 h in vitro, and the cells were cultured for 6 days in the absence of antigen before antibody production was measured. I asked Dr. Tadamitsu Kishimoto, then a postdoctoral fellow who had been working on the structure of IgM in Japan, to take on the challenge of this new project.

Fortunately, we were able to make progress. First, we showed that depletion of cells expressing surface IgG ($SIgG^+$ cells) and/or $SIgM^+$ from the lymph node cells diminished the secondary IgG

antibody response in vitro but did not affect the IgE antibody response. This finding indicated that B cells generating the secondary IgE antibody response were different from B cells mediating the IgG/IgM antibody responses with respect to their surface immunoglobulin (25). We also found that carrier-specific T cells obtained by supplemental immunization with a secondary carrier, adsorbed to aluminum hydroxide gel, collaborated with the hapten-specific IgE–expressing B cells to induce the secreted IgE antibody response, whereas carrier-specific T cells obtained by supplemental immunization with the same carrier protein in complete Freund's adjuvant collaborated with B cells for an IgG antibody response but failed to collaborate with IgE^+ B cells for IgE antibody production. The results indicated the importance of adjuvants and that carrier-specific T cells for the secondary IgE antibody response were distinct from those for the IgG antibody response (26). Subsequent studies revealed that stimulation of carrier-specific T cells with the carrier resulted in the formation of the soluble factors (cytokines) and that the cytokine involved in the differentiation of hapten-specific IgE^+ B cells into IgE antibody–forming cells was different from the cytokine for the differentiation of IgG B cells into IgG-forming cells (27).

Dr. Kishimoto continued the research, using the in vitro culture system. After he went back to Japan, he reproduced the findings on rabbit lymph node cells in mice and then characterized IL-6, the T cell–derived cytokine important for the IgG antibody response. Subsequently he succeeded in the molecular cloning of the gene encoding IL-6 and identified the IL-6 receptor (IL-6R). Unexpectedly (for me), their subsequent research revealed an important role for the IL-6–IL-6R system in rheumatoid arthritis (28). Although the research was carried out after he went back to Japan, their findings pleased Professor Harvey, who had predicted an important role for immunological mechanisms in the pathogenesis of rheumatoid diseases.

At Johns Hopkins, we continued our studies on the IgE antibody response in the mouse. One of the important findings obtained at that time, in collaboration with Professor T. P. King, who had isolated ragweed antigen E, was the identification of the T cell epitope in the α polypeptide chain of the antigen (29). This was the first evidence indicating that the T cell epitope was different from the B cell epitope in a natural protein antigen. We wondered if stimulation of antigen-specific T cells with urea-denatured antigen, which contained the T cell epitope but not the B cell epitope in the native antigen, might induce the generation of suppressor T cells (now known as regulatory T cells) that regulated the IgE antibody response to the native antigen. Retrospectively, our studies on the regulation of the IgE antibody response, followed by characterization of CD23, a low-affinity IgE receptor on B cells (30), failed to establish an effective method for isotype-specific regulation of the IgE antibody response. Nevertheless, studies on the IgE antibody response, together with analysis of IgE-dependent mediator release from mast cells/basophils, were quite useful for training postdoctoral fellows.

Another research project in our laboratory at Johns Hopkins was elucidation of the molecular mechanisms of IgE-mediated allergic reactions. Identification of basophils and mast cells as the target cells for IgE raised the possibility that cross-linking of cell-bound IgE antibody molecules by antigen might facilitate dimerization of the IgE-bound receptor molecules on the surface of mast cells and that cross-linking of the receptor molecules might be responsible for triggering the biochemical pathway leading to histamine release from these cells (31). Fortunately, Dr. Henry Metzger at the NIH became interested in the IgE Fc receptor (FcεR), and he obtained rat basophilic leukemia (RBL) cells and a rat immunocytoma that produced rat IgE. He used these reagents to begin to analyze the structure of the FcεR on RBL cells. Based on his findings, we tried to prepare rabbit antibodies specific for the FcεR molecules on RBL cells. RBL cells were saturated with rat IgE obtained from the IgE-forming rat immunocytoma, and the IgE-receptor complexes in the lysate of the RBL cells were precipitated with rabbit anti–rat IgE. A rabbit was immunized repeatedly with the IgE-receptor complexes, and the antibody specific for the receptor molecule in

the rabbit antiserum was purified by using RBL cells as the immunosorbent (32). Fortunately, the antibody obtained by this procedure was specific for the α chain of the high-affinity IgE receptor (FcεRI), and the antigenic determinant reacting with the antibody was in the IgE binding site of the α chain (33). As expected, the anti-receptor antibody, as well as the $F(ab')_2$ fragment of the antibody, induced histamine release from normal rat mast cells and caused an immediate skin reaction in normal rats, but the reaction of the antibody was inhibited if the receptors on the mast cells had been saturated with rat IgE. It was also found that the monomeric Fab′ fragment of the anti-receptor antibody blocked passive sensitization of mast cells with IgE antibodies; however, the Fab′ fragment failed to induce the skin reaction or histamine release from mast cells. The results indicated that the antibodies were specific for the IgE-combining site in the FcεRI, and that cross-linking of FcεRI was responsible for the induction of histamine release from mast cells by antigen-IgE antibody reactions (34).

The next question concerned the biochemical pathway for IgE-mediated histamine release. However, RBL cells coated with IgE failed to release histamine upon challenge with anti-IgE. Thus, Terry tried to develop cultures of normal rat mast cells. Fortunately, she succeeded in obtaining normal rat mast cells by long-term (>40 days) culture of rat thymus cells on an embryonic fibroblast monolayer (35). The average number of IgE receptors per cultured mast cell was 100,000–400,000, and the equilibrium constant of the binding reaction between the receptor and IgE was in the order of 10^8 M^{-1}, about tenfold less than the equilibrium constant for the binding of IgE to the receptors on peritoneal mast cells. Because the number of FcεRI/cell and the equilibrium constant between IgE and FcεRI on the cultured mast cells increased during the culture, the mast cells obtained by 40-day culture might not have reached full maturation. Nevertheless, cultured mast cells could be sensitized with IgE antibody, and the sensitized mast cells released histamine upon challenge with antigen (36).

Based on these findings, we also succeeded in the selective growth of human basophilic granulocytes in suspension cultures of mononuclear cells from umbilical cord blood (37). The histamine content of human basophils obtained from the cultures was comparable to that of basophils from peripheral blood. As expected, the basophils developed in the culture could be sensitized with human IgE, and the sensitized basophils released histamine upon challenge with anti-IgE.

Finally, Terry made an attempt to obtain human mast cells by long-term culture of mononuclear cells from umbilical cord blood on 3T3 fibroblast monolayers, and she succeeded in developing human mast cells (38). Subsequent studies revealed that stem cell factor (c-kit ligand) formed by the 3T3 fibroblasts was responsible for the development of mast cells in the cultures (39). Mast cells obtained after culture of mononuclear cells with c-kit ligand expressed FcεRI and could be sensitized with human IgE for anti-IgE-induced release of histamine and leukotriene C4. However, analysis by electron microscopy showed that the mast cells obtained in this culture system were immature, as compared with those obtained by culture of mononuclear cells with 3T3 fibroblasts. Finally, Dr. H. Saito, who was Terry's postdoctoral fellow at Hopkins, established the method for generating mature human mast cells by long-time culture of cord blood mononuclear cells in the presence of c-kit ligand, IL-6, and prostaglandin E_2 (40). Availability of pure human basophils and mast cells by long-term culture of umbilical cord blood mononuclear cells facilitated the analysis of the biochemical pathway for the degranulation of the target cells of IgE, leading to allergic reactions (41).

MY TASKS AS A MEMBER OF THE SCIENTIFIC COMMUNITY

Several months after we moved to Johns Hopkins, in 1970, I was asked to become a member of an NIH study section. The NIH asked me to attend a study section meeting to see how these

grant applications were being evaluated. Discussion by the members of the study section was quite impressive, and it was obvious that the evaluation of research proposals is one of the most important tasks of scientists. Therefore, I agreed to the NIH request and served as a member of the Allergy and Immunology Study Section for four years. Unfortunately, the Nixon administration severely cut the NIH's budget, and as a result, the number of funded research grant applications diminished to 10% or less of the total number evaluated. In these circumstances, it became quite difficult for young investigators to obtain funding. When I tried to recommend the unique proposals of young investigators, I was criticized. However, I still remember the beautiful presentations and fair evaluations made by Professors Jonathan Uhr, Hugh McDevitt, Dick Dutton, and others during the study section meetings.

The National Institute of Allergy and Infectious Diseases (NIAID) also asked me to join the Allergy and Clinical Immunology Committee for the evaluation of applications for program project grants and training grants, and I served in this capacity from 1979 until 1983. Professor Daniel Nathans, then Director of the Department of Microbiology at Johns Hopkins, was awarded the Nobel Prize in Physiology or Medicine in 1978, together with Drs. Werner Arber and Hamilton Smith. Afterward, he established the Department of Molecular Biology and Genetics. The Dean of the Medical School established an immunology training program and asked me to take the responsibility of setting up an immunology course for medical students. Because the lectures designed for medical students were not sufficient for training immunologists, we applied for an NIH training grant to establish a PhD course in immunology as well. Fortunately, outstanding scholars such as Dr. Bill Paul from the NIH and Professors Merrill Chase and Henry Kunkel from Rockefeller University were willing to give lectures in this course. Graduate students of not only immunology but also biochemistry, pharmacology, and hygiene attended our series of lectures in immunology.

In 1979, I was recommended as a candidate for election to the Council of the American Association of Immunologists (AAI). Since I did not have American citizenship, I hesitated before agreeing to run. But I agreed, and to my great surprise, I was elected. I served as AAI President from 1984 to 1985. In my presidential address, I included my personal philosophy, "You may compete with your competitors; however, you should keep in mind that your competitors are the persons who recognize and greatly appreciate the value of your research. To preserve our scientific society, collaborating with your competitors is much more important than defeating them."

Another important responsibility as a member of the scientific community is to develop the careers of young scientists. The total number of postdoctoral fellows who had trained in our laboratories at CARIH, at Johns Hopkins, and later at the La Jolla Institute of Allergy and Immunology (LIAI), was only 50. However, 25 of them became professors in medical schools and/or heads of departments at prominent institutions in the United States, Japan, and European countries. As most of them focused on new projects after they left our research groups, their successes clearly were the results of their own great efforts. Nevertheless, I am very pleased that we could contribute so meaningfully to the development of the next generation of investigators.

WHY WE MOVED TO LA JOLLA

In the 1980s, the US economy declined and the budget for NIH diminished. Because 55% of the total running cost of the Johns Hopkins Medical School depended on grants from the government, a decrease in grants with the associated decrease in funding for indirect costs affected the operations of the entire medical school, while the lack of research funds affected the morale of some faculty members. Some individuals who did not have any experience in basic research reported incorrect information to the Dean, and we lost the favorable environment for conducting basic research in the medical school at that time.

At Johns Hopkins School of Medicine, department directors customarily stepped down at the age of 65. I decided to follow the custom and began to look for a candidate who would take care of the immunology program after my retirement. Fortunately, Dr. Douglas Fearon at Harvard was interested in taking the job, and he became director of the immunology program in 1988.

Now that my successor was decided, Terry asked me to retire and to move back to her native city in Japan. During the Federation meeting in 1988, however, Dr. Makoto Nonaka, who worked with Dr. David Katz at the Scripps Research Institute, told me that he had been looking for Japanese companies that would give financial support to establish a nonprofit institute for immunology in La Jolla, California. Several months later, he called and told me that a Japanese brewery company, Kirin, was willing to donate $40 million to establish the institute. Thus, he was planning to sign a written contract with an executive of the company in Baltimore and asked me to join the meeting and confirm the contract.

After the meeting, a Kirin executive asked me to become Scientific Director of the new institute; however, I did not accept the offer. As the financial basis of the institute was contributions by a for-profit company, I was afraid that the California state government might have doubts about the purpose of the research organization and might not approve the institute as a nonprofit organization. If it did not, scientists at the institute would not be allowed to apply for NIH research grants. Under these conditions, I would not be able to recruit excellent basic scientists to the institute.

I visited La Jolla to see the Dean of the University of California San Diego (UCSD) School of Medicine, Dr. Gerard Burrow, and Dr. Frank Dixon of the Scripps Research Institute. Both the Dean of the School of Medicine and Dr. Dixon were very pleased to have the institute in La Jolla, and the Dean suggested affiliation of the institute with UCSD. He offered joint appointments at UCSD for both Terry and me and asked us to be involved in teaching graduate students. Drs. Burrow and Dixon also were willing to become members of the Board of Directors of the new organization. As I then had confidence that LIAI would be accepted by the state of California as a nonprofit research organization, I began to recruit young scientists who would be able to establish their own research groups at the institute. We had funds sufficient for equipping their laboratories, and to support their research for two years. I believed that we could establish the circumstances in which they could focus their efforts on their research without worrying excessively about funding. In October 1989, Dr. Nonaka recruited administrative staff, moved into the new building of LIAI, and set up the administrative office. We moved to La Jolla in November, together with our postdoctoral fellows from Hopkins, and began to set up our laboratories.

However, Dr. Nonaka appeared to have difficulties in establishing the administration of the institute. It was a great surprise for me when he resigned two years after we opened the institute and the board members asked me to take the position of President, together with Scientific Director. Although I did not have interest in taking an administrative job, there was no other choice for maintaining LIAI. There were some difficulties. I had to replace the chief of administration and it took one month to reestablish administration of the institute. A lawyer was involved in the preparation of the policies of the institute and contract documents.

Dr. Dixon frequently visited LIAI to give me advice (**Figure 7**). He also was willing to participate in the social activities of LIAI. Looking back, it would have been impossible to establish LIAI without his advice. After one year, some visitors looked around the institute and mentioned, "This institute is a paradise for young investigators!" I am very pleased that my dream has succeeded and has materialized under the leadership of my successors, Drs. Howard Grey and Mitchell Kronenberg. Because of their efforts and leadership, LIAI developed into an institute of worldwide renown.

Figure 7

Drs. Teruko and Kimishige Ishizaka with the late Dr. Frank J. Dixon (1920–2008) and Marion Dixon (1919–2015).

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The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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