Identification of IgE and the Mechanisms of Allergy

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I feel very honoured to have been chosen to receive 2000 Japan Prize for research in Host Defence. With so many discoveries made in the field of Immunology and infectious diseases deserving recognition, I am indeed fortunate to have been chosen to accept this award. I am particularly pleased recognition of allergy research that has been a minor speciality in the field of Immunology.

Hitorical Background

About 50 years ago, many clinicians believed that allergy is caused by an idiosyncrasy. Some people seriously considered that allergy is a psycological disease. However, many immunologists anticipated that immunological mechanisms may be involved in allergy, because allergic individuals showed erythema-wheal type skin reaction upon injection of the allergen, to which they were sensitive. Indeed, the presence of skin-sensitizing activity in the serum of allergic patients was demonstrated in 1921 by Prausnitz who had passively sensitized his forearm by an intracutaneous injection of serum from his colleague, Kustner. The skin site receiving the serum gave an erythema-wheal reaction upon challenge with fish extract to which Kustner was sensitive. The serum substance responsible for the passive transfer was called "reagin", but the nature of this substance remained unknown for the next 40 years.

In 1950s, many immunochemists and protein chemists were interested in the nature of reagin. They fractionated sera of allergic patients by various methods, and determined the distribution of reaginic activity in the fractions. These studies indicated that the physicochemical properties of reagin are slightly different from those of usual antibodies.

At the beginning of 1960s, it became clear that proteins with antibody activities are heterogeneous in terms of their physicochemical properties and antigenic structure, and the concept of immunoglobulin was established. Thus, immunochemists suspected that reaginic activity may be associated with a minor class of immunoglobulins. At this point, Heremans from Belgium, who had originally established IgA, isolated IgA from reaginic sera by his method, and recovered the reaginic activity in the IgA fraction in which no serum protein other than IgA was detectable. As their results were reproduced by several investigators, including ourselves, reagin was considered to be IgA antibodies in 1963 to 1965.

However, conflict to the IgA hypothesis was obtained in our experiments on human isoagglutinins. We immununized type B and type O normal individuals with blood group A substance, and isolated IgG, IgA and IgM in the antisera. Each of the immunoglobulin fractions contained a lot of anti-A isoagglutinins, but none of them could sensitize normal human skin for Prausnitz-Kustner(P-K) reaction even when 1 μg of the antibody was injected into a skin site for passive sensitization. As I believed that biologic activities of antibodies are decided by the Fc portion of antibody molecules, I could not accept the idea that IgA antibodies against allergens have reaginic activity, while IgA isoagglutinin does not, and thought about the possibility that reaginic activity might be associated with an impurity in the IgA fraction. Thus, we determined as to whether the precipitation of IgA in the fraction with anti-IgA antibodies might remove reaginic activity in the fraction. The results clearly showed that the reaginic activity of the IgA fraction from allergic patient's sera did not diminish after complete removal of IgA, indicating that the reaginic activity is associated with an impurity rather than IgA itself.

Identification of IgE:

Our findings on the reaginic activity in the IgA fraction of patient's sera created more problems than answers. After the removal of IgA in apparently pure IgA fractions, the concentration of total human immunoglobulin in the supernatnats was in the order of 1 μg/ml. Yet, the P-K titer of the supernatant was almost comparable to the P-K titer of the original serum,

from which the IgA fraction was obtained. The results suggested that the concentration of the carrier protein of reaginic activity in the original serum is in the order of microgram/ml. If this is the case, failure of identifying the carrier protein of reaginic activity by previous investigators may probably be due to low concentration of the protein, which was unpredictable from the concentration of the other immunoglobulin isotypes. Nevertheless, our prediction of low concentration of the carrier protein forced us to switch our strategies from isolation to biochemical identification of the protein, because a sufficient amount of the protein for biochemical characterization can not be isolated unless several liters of patient's sera are available.

Our approach was to prepare polyclonal antibodies specific for reagin, and to biochemically identify the protein by using the antibodies. For this purpose, we repeatedly immunized rabbits with a reagin-rich fraction, and absorbed the antisera with IgG and IgA. After absorptions, the antisera did not give a precipitin band with any of the known immunoglobulins nor with normal human serum, but one of the antisera could absorb reaginic activity in sera of allergic patients. Indeed, we found that the antiserum precipitated a γ1 globulin in the reagin-rich fraction of the serum of a ragweed-sensitive patient. Furthermore, the γ1 precipitin band specifically bound radio-labeled ragweed antigen, indicating that the γ1 globulin has antibody activity. As the rabbit antiserum did not contain any antibodies reacting with a known immunoglobulin, the γ1 globulin must be a unique immunoglobulin. Thus, we tentatively called this protein γE, because I believed that this immunoglobulin can induce erythema-wheal reactions. When reaginic sera were fractionated by ion-exchange column chromatography, gel filtration, sucrose gradient ultracentrifugation and gel electrophoresis., complet correlation was obtained between the distribution of γE antibody and reaginic activity.

Based on the information obtained in the experiments, attempts were finally made to isolate γE in the serum of ragweed-sensitive patients. The final preparation did not contain a detectable amount of known immunoglobulin, such as IgG, IgA, IgM or IgD, but contained γE, and the γE bound radio-labeled ragweed antigen. As this preparation gave a positive P-K reaction at a dilution of 1: 80,000, we concluded that γE is the carrier protein of reaginic activity. As expected, γE had unique antigenic determinants, which are lacking in the known immunoglobulins, and shared κ and λ light chain determinants with the other isotypes, suggesting that γE represents a unique immunoglobulin isotype. Subsequently, a myeloma protein with the same antigenic structure as γE was found. Thus, γE was officially designated IgE.

Target cells for IgE:

When IgE was identified, however, many immunologists and allergists were suspicious about possible role of IgE antibodies in allergic diseases. Thus, we tried to obtain evidence that IgE antibodies can sensitize not only the skin but also lung tissues. Monkey lung fragments incubated overnight with serum of a ragweed sensitive individual released both histamine and leukotriene upon challenge with ragweed allergen. However, the sensitizing activity of the reaginic serum was completely removed by absorption of the serum with anti-IgE immunosorbent. When the same lung fragments were sensitized with the absorbed serum, neither histamine nor leukotriene was released upon challenge with the antigen. Thus, the results showed that the IgE antibodies were responsible for sensitization of lung tissues, and were involved in the release of the chemical mediators which were believed to cause allergic symptoms.

Nevertheless, an essential role of IgE antibodies in allergic diseases was not appreciated until we found receptors for IgE on basophil granulocytes and mast cells. When leukocytes were incubated with radio-labeled anti-IgE in the presence of EDTA, which

prevented histamine release, essentially all basophilic granulocytes were radio-labeled, while none of the neutrophils, eosinophils, lymphocytes and monocytes bound the antibody. Selective binding of IgE to basophils was confirmed by incubating radio-labeled E myeloma protein with leukocytes. In order to identify tissue cells which bear IgE, E myeloma protein was injected into monkeys, and a lung cell suspension was treated with radio-labeled anti-IgE. Anti-IgE bound to mast cells but not to the other cells. When the same cell suspension was treated with radio-labeled anti-IgG, the antibody bound to macrophages but not to mast cells, indicating that mast cells bear specific receptors for IgE. As expected, incubation of the lung cell suspension with anti-IgE resulted in the release of histamine and leukotriene, while anti-IgG failed to induce the mediator release. These findings indicate that the reaction of anti-IgE with IgE present on mast cells induces the relaease of the chemical mediators, but IgG-anti-IgG reaction on macrophages do not. Since almost all histamine in the lung cell suspension is associated with the granules of mast cells, it was obvious that antigen-IgE antibody reaction on mast cells initiated enzymatic sequences leading to degranulation.

An important characteristics of the binding between IgE and IgE-receptors is high affinity. In both the rodent mast cell system and human basophil system, the equilibrium association constant of the binding is in the order of 10^9 to $10^{10}M^{-1}$. Such a high affinity of IgE for the receptors on mast cells explains why a minute dose of IgE antibody could sensitize homologous tissues. One of the characteristic properties of reaginic antibodies was that passive sensitization persists for a long time. This property may be explained by low dissociation constant between IgE and the receptors.

Molecular mechanisms of IgE-mediated allergic reactions:

A question to be asked at this point was why only IgE, but no other immunoglobulin isotype, could sensitize mast cells? As expected, the Fc fragment, but neither the Fab nor $F(ab')_2$ fragment, of an E myeloma protein bound to mast cells. Indeed, the affinity of the recombinant Fc fragment of human IgE for human basophils was comparable to the affinity of an E myeloma protein for the same cells. Further studies indicated that the binding site of IgE to the receptors is the CH_3 domain of heavy(ε chain of IgE molecules.

On the other hand, IgE receptors on mast cells, called FcεRI, were biochemically characterized by Henry Metzger and his associates. The receptors are composed of three kinds of polypeptide chains, called α, β and γ chains. Molecular cloning of the polypeptide chains revealed amino acid sequences of the polypeptides. As expected from the structure of the three polypeptide chains, IgE binds to the α chain, while β and α chains appear to play roles in transduction of the signal from the α chain to intracellular enzymes. It became also clear that CH_3 domain of IgE binds to the α2 domain of the α chain.

The next question was the mechanism through which the reaction of either anti-IgE or allergen with mast cell-bound IgE induced mediator release. It became clear that cross-linking of cell-bound IgE with divalent anti-IgE or multivalent antigen was required for activation of mast cells. Neither the monovalent hapten nor Fab fragment of anti-IgE induced histamine release. As IgE molecules are bound to FcεRI with high affinity, we anticipated that cross-linking of IgE may bring the two receptor molecules in close proximity, and that the dimerization of the receptors may be responsible for activation of mast cells. Indeed, rabbit antibodies against the receptors induced histamine release. The Fab fragment of the anti-receptor antibodies bound to the receptors, but failed to induce histamine release. However, if one cross-linked the receptor-bound Fab fragment with anti-rabbit IgG, histamine relase was observed. These findings collectively show that dimerization of the receptors, rather than

dimerization of IgE, was responsible for IgE-dependent mediator release. However, only the molecules having high affinity for the receptors are IgE. Under the physiological conditions, therefore, cross-linking of the receptors occur only through IgE molecules.

It is reasonable to speculate that dimerization of FcεRI induces the activation of membrane-associated enzymes. Indeed, evidence has been accumulated that the cross-linking of the receptors induces the activation of tyrosine kinases, which in turn phosphorylate phospholipase C. The activated phospholipase C hydrolyses phosphatidyl 4,5 diphosphate to form diacylglycerol and inositol 1,4,5-triphosphate, latter of which functions as a second messenger for mobilization of intracellular calcium. Considering that Ca^{2+} influx into mast cells by Ca-ionophore induces degranulation, we believe that such a biochemical pathway for increasing intracellular Ca^{2+} is involved in degranulation. It is also known that cross-linking of FcεRI induces activation of phospholipase A_2 which in turn hydrolyses phospholipid for the formation of arachidonic acid. We believe that this process is an essential step for the synthesis of leukotriene C4 and prostaglandine D2, which are derivatives of arachidonic acid, and cause allergic symptoms.

Regulation of IgE antibody response:

The definitive role of IgE antibodies in allergic reactions encouraged the studies on the regulation of the IgE antibody response. An important fact in the IgE antibody response in experimental animals is that the IgE antibody formation is obtained under limited experimental conditions. In the mouse, the persistent IgE antibody response is obtained only when a high responder strain was immunized with a minute dose of a potent immunogen together with an appropriate adjuvant. In atopic patients who are sensitive to ragweed allergen, however, the serm concentration of IgE antibodies against the allergen was comparable to that of IgG antibodies, and higher than the IgA antibodies, as determined by antigen-binding activity. Considering that the concentration of the total IgG in the sera is 10,000 fold higher than that of the total IgE, the results indicate the existence of a mechanism, through which the antibody response to allergens in atopic patients becomes preferential for the IgE isotype. We did not know the mechanism for a long time, however, progress made by the other investigators strongly suggest that the peculiar profile of the antibody response to allergens in atopic patients must be due to helper T cells involved. It is well known that differentiation of B cells and isotype switch are controlled by helper T cells, which collaborate with the B cells, and the cytokines produced by the helper T cells. In 1986, Tim Mossman and his associate have shown that mouse helper T cell clones can be classified into Th1 and Th2 types in terms of the profile of cytokines produced upon activation, and that Th2 cells, which produce IL-4 and IL-5, are required for the development of IgE-forming cells. Essential roles of IL-4 for the IgE synthesis were confirmed by the deletion of the IL-4 gene in the mouse. The IL-4-gene deficient mice produce IgG but no IgE antibodies.

This principle may probably apply for the human system. Although many human helper T cells are Th0 type, which form both IL-4 and IFNγ upon activation, the majority of allergen-specific T cell clones established from peripheral blood of allergic patients produce IL-4 upon antigenic stimulation, and the number of Th1 type clones, which form IFNγ but no IL-4, was quite limited. In contrast, most of the tuberculin-specific helper T cell clones and tetanus toxoid-specific helper T cell clones established from the same patients are Th1 type, that form IFNγ but no IL-4 upon antigenic stimulation. The results correlated with the fact that immune system of these pateints form IgE antibodies against allergen but do not form IgE antibodies against tuberculin or tetanus toxoid. The proportion of Th1 cells to Th2 cells in antigen-specific T helper cell population may explain why allergic patients form the IgE antibodies against allergens.

Why the majority of allergen-specific helper T cells became Th2 type? An important principle in this respect is that the Th1 and Th2 type helper cells develop from a common precursor. Many factors affect the direction of the differentiation from the naive T cells either to Th1 or to Th2. When the dose of immunogen for the antigenic stimulation of the precursor cells is minimum, the cells preferentially differentiate toward Th2. It also seems likely that the nature of the antigen-presenting cells involved in the antigenic stimulation may affect the direction of the differentiation of the naive T cells. When we are exposed to allergens, the antigen goes through respiratory tract or skin, and the dose of allergen reaching the lymphoid tissues is quite limited. Such a route of the antigen administration may probably create conditions favorable for the Th2 development. In fact, evidence has been presented that immunization of mice by patching ovalbumin to their skin induced the development of Th2 type helper cells, and that the epicutaneous immunization was quite favorable for the production of IgE antibodies.

Another important aspect on the differentiation of helper T cells is that cytokines present in the environment of the precursor cells determine the direction of the differentiation of the cells upon antigenic stimulation. If the naive T cells are stimulated by antigen in the presence of IL-12, these cells differentiate towards Th1, while the stimulation of the same precursor T cells in the presence of IL-4 facilitates the development of Th2 cells. Indeed, such an effect of cytokines on the direction of the differentiation may explain the clinical observations that allergic patients are sensitive to a single allergen to begin with, but they become allergic to multiple allergens. When the IgE antibodies against one allergen are being formed, Th2 cells specific for the allergen are being stimulated and form IL-4. If the second allergen is introduced into the same lymphoid tissues, when the IgE antibody response to the first allergen is in progress, IL-4 is present in the environment of the naive T cells specific for the second allergen. In this situation, the naive T cells specific for the second allergen will differentiate toward Th2, and these cells facilitate the formation of IgE antibodies to the second allergen.

Mechanisms of Allergic Inflammation:

In the past 15 years, so-called late phase response in allergic diseases called much attention. In allergic broncheal asthma, exposure of patients to allergen immediately induces respiratory symptoms, which could be explained by chemical mediators released from mast cells by IgE-dependent mechanisms. However, many patients suffer from more severe respiratory symptoms 6 to 8 hours after the exposure to allergen. It is now known that the late-phase response is caused by allergic inflammation, and the patients show broncheal hyperreactivity to non-specific stimulus.

When IgE was established, the cause of allergic inflammation was considered to be independent of IgE. However, an injection of a minute dose of anti-IgE into the skin of allergic patients induced not only an immediate erythema-wheal type reaction, but also local inflammation several hours after the injection, suggesing that IgE/mast cells are involved in the induction of the late phase response. Subsequent studies revealed that cross-linking of mast cell-bound IgE either by allergen or by anti-IgE induced not only the release of chemical mediators, such as histamine and leukotriene, but also the synthesis of cytokines, such as TNFα and IL-5 by the cells. One of the characteristic properties of allergic inflammation is infiltration of eosinophilic granulocytes. IL-5 activates eosinophils, cause eosinophilia, and participates in the infiltration of eosinophils into the locus. Once eosinophils are activated, these cells produce platelet activating factor (PAF) and leukotriene, which induce bronchoconstriction. Furthermore, activated eosinophils release a variety of cytokines and proteins that are potentially important in the allergic response.

Among them, eosinophilic cationic protein and major basic protein are cytotoxic to the airway epithelium, and lead to airway hyperresponsiveness(AHR) to nonspecific stimulus.

However, the mechanisms of allergic inflammation appears to be more complicated. Examination of broncheal lavage fluids and skin tissues of atopic dermatitis demonstrated many cells containing IL-5 mRNA, the majority of which were T cells. Thus it appears that the major cell source of IL-5 is Th2 cells rather than mast cells.

Analysis of allergic inflammation requires animal models of allergic diseases. Recently, AHR: a characteristic symptom of broncheal asthma, could be reproduced in experimental animals, such as dogs and mice. In these systems, animals are actively immunized with an antigen together with an appropriate adjuvant for the production of IgE antibodies, and receive bronchoprovocations by inhalation of aerosolized antigen. Intravenous injections of graded dose of acetylcholine to the animals clearly showed that the immunized and aerosolized animals exhibit broncheal hyperresponsiveness to acetylcholine and allergic inflammation in the airways.

Concerning the mechanisms for the development of AHR, evidence has been presented that antigen-specific Th2 cells, which play essential roles in the production of IgE antibodies, are involved in the allergic inflammation. However, our recent experiments indicated that IgE/mast cell system are also involved in the development of AHR. We employed the mast cell-deficient W/W^v mice in comparisons with their congenic littermate, +/+ mice. After immuniztion and inhalation of aerosolized antigen, no significant difference was observed between the two strains in the magnitude of the IgE antibody response and eosinophil infiltration into airways. The +/+ mice showed AHR to acetylcholine, whereas the W/W^v mice failed to do so. Possible roles of mast cells in the development of AHR was confirmed by the transfer of mast cells derived from the bone marrow of +/+ mice. The W/W^v mice receiving the mast cells developed AHR, similar in extent to that observed in +/+ mice. In this system, the major cell source of IL-5 is Th cells. However, IgE/mast cells appear to play an important role in the development of broncheal hyperresponsiveness.

Summary:

Human IgE is the only immunoglobulin isotype that has affinity for mast cells and bind to the cells through FcεRI. Cross-linking of the mast cell-bound IgE antibodies by multivalent antigen triggers the cells for the release of histamine and formation of derivatives of arachidonic acid, which cause allergic symptoms. This mechanism explains why IgE antibodies cause type I allergy such as pollinosis. Evidnce was also obtained that both IgE/mast cell system and Th2 cells, which play a key role in the IgE antibody response, are involved in the development of allergic diseases such as broncheal asthma. We expect that elucidation of the mechanisms of allergic diseases will provide several approaches to control allergic diseases.

Discovery and characterization of IgE facilitated the analysis of the mechanisms of allergic reactions. Subsequent, extensive studies by many investigators extended the basic findings to the mechanisms of allergic diseases. It is my understanding that our work was recognized because of the development of allergy research by the other investigators. I am greatly honored to represent these investigators and accept the Japan Prize.