ADVANCES IN

Immunology

VOLUME 23

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Immunology

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VOLUME 23
1976

ACADEMIC PRESS *New York San Francisco London*
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 61-17057

ISBN 0-12-022423-2

PRINTED IN THE UNITED STATES OF AMERICA

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PREFACE

The familiar and somewhat tiresome debate over the relative merits of fundamental versus applied research has if anything intensified in the last few years. This has occurred largely as a result of the greatly increased competition for funds that exists today. It has been fostered to a considerable degree by the "somewhat snobbish attitude of many academics to applied research." The distinction is purely arbitrary; scientific knowledge is a continuum in which every component part can and does feed back on every other. Nowhere is this more clearly apparent than in the field of immunology, as exemplified by the articles in Volume 23.

The first paper is by Dr. Kimishige Ishizaka, the individual primarily responsible for the basic work on IgE antibodies and their role in reaginic hypersensitivity. The initial definitive work was carried out in the human system, and the extension to the cellular regulation of IgE antibodies, the main topic of the review, was continued in various experimental animals. The important role of both helper and suppressor T cells in this regulation is quite apparent. It is still uncertain whether the same cells are involved as those defined for the major immunoglobulin classes. Promising approaches to therapy derived from the animal-model work are discussed.

The work of Dr. T. P. King, author of the second article, has centered on the chemistry of the allergens, a subject which has advanced markedly in the last few years, largely through his efforts. Ragweed pollen allergens have received the most attention, and antigen E, the dominant antigen involved in hypersensitivity, has been isolated and characterized in considerable detail. It consists of two non-identical polypeptide chains with molecular weights of approximately 26,000 and 13,000. Additional ragweed allergens have been isolated, but their significance relative to antigen E remains to be defined. Many other types of allergens have been isolated as well. Of special interest is the current active work on the chemical modification of these isolated proteins for possible therapeutic immunization.

The third article is written by Drs. Dupont, Hansen, and Yunis, and deals primarily with the new and exciting developments in MLC typing in human histocompatibility studies. These workers have played a major role in placing this system on a firm scientific basis. The use of homozygous cells from specific individuals has made it possible to delineate at least six different distinct MLC antigens, and there are clearly more. Some of these can also be recognized by B-cell-specific alloantisera and clearly relate to the Ia antigens of the murine system. It is of special

interest that certain disease associations, as well as the genes involved in certain of the complement components, appear more closely linked to the MLC genes than to the other components of the HLA system.

The last paper covers the somewhat neglected area of the immunology of lipids and glycolipids. The authors, Drs. Marcus and Schwarting, have had wide experience in this field and their contributions have played a major role in current recognition of the significance of these antigens. Suddenly, with the great expansion of interest in cell membranes, the glycolipids have assumed a particular importance and their study by immunological procedures as specific moieties of the cell membrane is receiving great emphasis. Much remains to be learned about the many different types of lipid antigens and their cross reactions, but this review provides the many interested investigators with an up-to-date treatment of the subject.

HENRY G. KUNKEL
FRANK J. DIXON

CONTENTS

Cellular Events in the IgE Antibody Response[1]

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I. Introduction

Since the discovery of IgE in the serum of hay fever patients (45), much progress has been made in the field of reaginic hypersensitivity. It is now established that reaginic hypersensitivity reactions in atopic diseases are mediated by IgE antibody [reviewed by Ishizaka and Ishizaka (53)]. Meanwhile, homocytotropic antibodies, which are similar to human IgE antibodies, were detected in experimental animals. Mota (109) and Binaghi *et al.* (11) first described production of rat "reaginic" antibodies after immunization with antigen plus *Bordetella pertussis* vaccine. Subsequently, antibodies that were capable of sensitizing homologous skin

[1] Supported by research grants AI-11202 from the U.S. Public Health Service, GB-41443 from National Science Foundation, and a grant from John A. Hartford Foundation. This is publication No. 223 from the O'Neill Laboratories at the Good Samaritan Hospital.

were found in rabbit (46, 91, 189), dog (133), mouse (110, 112, 130), monkey (47), guinea pig (88), pig (7), and cattle (39). The physicochemical properties of the reaginic antibodies in experimental animals are similar to those of human IgE, and their molecular sizes are distinct from those of immunoglobulins of the other isotypes. It was also found in each species that the antigenic structure of the immunoglobulin class to which the reaginic antibody belongs was different from IgG, IgA, and IgM. More recently, Bazin *et al.* (9) reported that the inbred Lou/WST rat strain presented a high incidence of spontaneous ileocecal immunocytoma, which secreted monoclonal immunoglobulins, and that nearly one-third of them represented a unique isotype to which reaginic antibody belonged. From the biological viewpoint, human IgE and reaginic antibodies in experimental animals share common characteristics. Once skin sites of homologous species are passively sensitized with the antibody, sensitization persists for 2 to 3 weeks. This property and the molecular size of reaginic antibodies are distinct from those of another type of skin-sensitizing antibodies that belong to a subclass of IgG.

A crucial role of IgE antibody in atopic diseases suggested that prevention or suppression IgE antibody formation is beneficial for atopic individuals. Identification of IgE antibodies in experimental animals provided an important tool for studying this problem. Fortunately, the scope of our knowledge on the mechanisms of antibody response has considerably broadened in the past decade [reviewed by Katz and Benacerraf (64)]. It is firmly established that collaboration of two distinct types of lymphocytes, i.e., bone marrow-derived precursors of antibody-forming cells (B cells) and thymus-derived lymphocytes (T

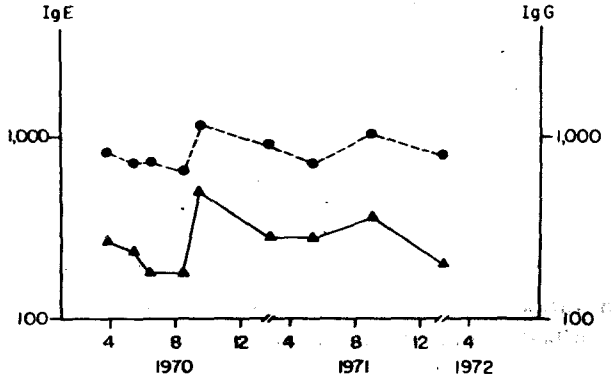

FIG. 1. Titers of IgE and IgG antibody in the serum of ragweed-sensitive patient. Both IgE (▲) and IgG (●) antibody titers are expressed by units. The IgE antibody unit corresponds to the minimal concentration of the antibody required to give a positive Prausnitz-Küstner reaction. [From Ishizaka and Ishizaka (44).]

cells), is essential for the induction of antibody responses to most protein antigens. This principle obtained with IgM and IgG antibody responses has been proved to be the case also in IgE antibody formation. From the immunological viewpoint, however, it became clear that IgE antibody responses in experimental systems have certain characteristic features that are not easily demonstrated in IgG antibody response. The purpose of the present review is to analyze the cellular events involved in the IgE antibody response in different experimental systems in comparison with the IgG antibody response. It is hoped that elucidation of the mechanisms for induction and suppression of IgE antibody response will provide a clue to future therapy for atopic diseases.

II. Immunoglobulin E Antibody Formation *in Vivo* and *in Vitro*

A. Kinetics of IgE Antibody Responses in Various Animal Species

Many years ago, Sherman *et al.* (142) followed reaginic antibody titers in hay fever patients by recording Prausnitz-Küstner reactions and showed that antibody titers persisted in the sera of ragweed-sensitive individuals. The results were recently confirmed by quantitative measurement of IgE antibody by a radioimmunoassay (RAST technique), which was developed by Wide *et al.* (185). Application of this method to measure serum IgE antibody in untreated ragweed-sensitive patients revealed that the antibody level persisted and that most patients showed secondary IgE antiragweed antibody responses after the ragweed season (Fig. 1) (44). Because the catabolic rate of IgE is very fast, with an average half-life of 2 to 3 days (178), persistence of IgE antibody titers in the sera of atopic patients indicates that IgE antibody is being formed continuously. Such a pattern of antibody formation, however, is not characteristic only for IgE. Titration of IgG antiragweed antibody in the sera of the same untreated patients by double antibody radioimmunoassay showed that IgG antibody formation also persisted, and the antibody titer definitely increased after the ragweed season. As shown in Fig. 1, the time course of IgG antibody produced to ragweed antigen E paralleled that of IgE antibody.

Several investigators injected allergen into non-atopic individuals in the course of their studies of hyposensitization treatment. Some normal individuals who received parenteral injections of alum-precipitated allergen developed IgE antibody against the allergen. The IgE antibody in the sera of these individuals disappeared within 2 to 3 months; however, many of them showed secondary IgE antibody responses after the

pollen season (99). De Weck (20) has shown a similar pattern of IgE antibody response in patients with hypersensitivity to penicillin. In many patients, the IgE antibody was detected when they had clinical symptoms but disappeared within several weeks after the administration of penicillin. Obviously, these patients will show secondary IgE antibody responses after reexposure to the drug.

Although the physicochemical properties and biological function are similar for IgE antibodies from various animal species, the kinetics of IgE antibody responses are different depending on the species and strains of the animals. Immunization of rats with usual protein antigens, such as ovalbumin (OA) (109) or human IgG (11) together with pertussis vaccine or aluminum hydroxide gel (alum) as adjuvants resulted in the formation of IgE antibody, but the antibody response was transient in nature. Maximum IgE antibody titer was reached at 10 to 14 days after the immunization and rapidly declined thereafter. A booster injection of the same antigen 4 to 5 weeks after the primary immunization did not elicit secondary IgE antibody response. Even when a secondary response was observed, maximum IgE antibody titer after a booster injection was lower than the maximum titer after primary immunization. As will be described later, the dose of antigen and nature of adjuvant employed for the primary immunization appear to be important factors in obtaining a secondary antibody response. By using a purified antigen from *Ascaris suum* extract (Asc-1), Strejan *et al.* (151) have shown a definite secondary IgE antibody response after a booster injection. More recently, Jarrett *et al.* (60) immunized Hooded Lister strain rats with 1 to 10 μg OA or keyhole limpet hemocyanin (KLH) together with 10^{10} pertussis vaccine and then gave a booster injection of homologous antigen without adjuvant at 30 days after primary immunization. This immunization schedule gave a definite secondary IgE antibody response. Other strains of rats, e.g., Sprague-Dawley, Wister, and Lewis, however, failed to show secondary IgE antibody response after a booster injection of antigen without adjuvant.

A unique system for obtaining an IgE antibody response in the rat was described by Tada *et al.* (155). Their immunization schedule was based on previous observations of Strejan and Campbell (148), who found that two closely spaced injections of *A. suum* extract (Asc) were effective in obtaining a high titer of reaginic antibody in the rat. Tada *et al.*, injected 1 mg of dinitrophenyl derivatives of *A. suum* extract (DNP-Asc) together with 10^{10} *Bordetella pertussis* vaccine into footpads of Wistar rats, followed by an intramuscular injection of 0.5 mg of DNP-Asc on day 5. In most animals, IgE antibody to homologous antigen was detected after the second injection. The IgE antibody titer reached a

maximum at 3 days after the second injection and declined thereafter. In their experiment, neither the first injection of antigen with pertussis vaccine nor the injection of antigen alone induced IgE antibody response. An average maximum antibody titer, which was determined by the homologous passive cutaneous anaphylaxis (PCA) reaction, was on the order of 1:80, and the antibody became undetectable at about 4 weeks. It was also found that a booster injection of the same antigen 4 weeks after the immunization failed to give a secondary IgE antibody response. Their immunization regimen is unique in that a large dose of antigen was used to obtain an IgE antibody response and that a single injection of antigen with pertussis vaccine failed to elicit the antibody response. As will be discussed later, usually a small dose of antigen is favorable for the IgE antibody response, and a single injection of an adequate dose of antigen with either pertussis vaccine or alum gives primary IgE antibody response.

Subsequently, Tada *et al.* (162) succeeded in sustaining the IgE antibody response by irradiation of rats with sublethal doses (200–400 R) of X-ray, 1 day before or 1 day after the initial injection of DNP-Asc with pertussis vaccine. In the irradiated rats, IgG antibody was undetectable, but serum IgE antibody titer was higher than that obtained in non-irradiated animals, and the titer was maintained more than 3 weeks. This immunization schedule was frequently used by Tada and his associates when they wished to analyze the mechanisms involved in the IgE antibody response. Unfortunately, irradiation abolished rather than sustained IgE antibody responses in some other strains such as Sprague-Dawley and Lewis (*see* Section V,C,1).

Rabbit IgE antibody was first described by Zvaifler and Becker (189) who had immunized animals with a relatively high dose of DNP–bovine γ-globulin (BGG) included in complete Freund's adjuvant (CFA). The antibody did not persist for long, and these animals failed to show secondary IgE antibody responses after a booster immunization. Subsequently, it became clear that immunization with a relatively small dose of antigen precipitated with alum (132) gave a primary IgE antibody response and that the animals immunized by this procedure frequently gave secondary IgE antibody responses after a booster immunization. In our experience, more than one-half of rabbits immunized with DNP-Asc showed secondary antihapten IgE antibody responses in which maximal antibody titers were higher than primary responses (48).

Mota and Peixoto (112) detected reaginic antibody in the mouse, after they had immunized outbred mice with a relatively high dose (50–100 μg) of antigen included in CFA, alum, or with pertussis vaccine. The IgE antibody response was transient in nature, and antibody became

undetectable in the sera within 3 weeks after the immunization. Similar results were obtained by Revoltella and Ovary (131) in several inbred strains of mice using DNP-KLH as antigen. Thus, the kinetics of reaginic antibody formation in the mouse were believed to be different from that observed in hay fever patients. Such a difference was overcome in a model developed by Levine and Vaz (90), who immunized several inbred strains of mice with 0.1–1.0 μg of protein antigens absorbed to alum. Repeated immunization at 4-week intervals resulted in a secondary response with a high titer of reaginic antibodies. Subsequently, Vaz *et al.* (173) succeeded in obtaining a persistent reaginic antibody response by injecting alum-absorbed OA (0.1 μg) into SW-55 strain mice. The reaginic antibody titer persisted for several months without booster injections. So far, the IgE antibody response in this system is the best model for reaginic antibody formation in humans. A persistent IgE antibody response has now been achieved with several different combinations of antigens and inbred strains of mice. For example, a minute dose of OA (0.05–0.2 μg) adsorbed to alum produced a persistent antibody response in DBA/1 and (C57Bl/6 $\times$ DBA/2)F_1 mice (176). Immunization of these strains with 1–2 μg DNP-KLH absorbed to 1–2 mg of alum gave a persistent anti-DNP antibody response (120). An injection of alum-absorbed ragweed antigen E into the A/J strain gave a similar pattern of IgE antibody response (52).

B. Helminth Infection and IgE Responses

It has been known for a long time that an intracutaneous injection of an extract of *Ascaris lumbricoides* into normal individuals frequently elicits a positive erythema wheal reaction, suggesting that IgE antibody is formed following *Ascaris* infection. Johansson *et al.* as well as others reported that total IgE levels in sera increased in most individuals infected with any one of a variety of helminths including *A. lumbricoides* (61), *Capillaria phillipinensis* (183), and *Ancylostoma* (6). Infected individuals' other serum immunoglobulins, such as IgG, IgM, IgA, and IgD, were usually in the normal range or were elevated only slightly emphasizing the strong relationship of helminth infections with IgE.

The IgE antibody formation following helminth infection was established in experimental animals such as the rat, mouse, and rabbit (108, 115, 118, 137, 190). Nematodes, cestodes, trematodes, as well as arthropods all share this immunogenic characteristic (117). A typical example was shown in the rat by Ogilvie (115), who demonstrated IgE antibody formation after infection with *Nippostrongylus brasiliensis* larvae. The IgE antibody against worm extract became detectable 3–4 weeks after the infection, and antibody persisted for a longer period of time than that

obtained by an artifical immunization with protein antigen included in an appropriate adjuvant. Furthermore, the animals showed a definite secondary IgE antibody response upon reinfection (116, 188). Recently, Jarrett and Bazin (58) determined total IgE levels in rats infected with *N. brasiliensis*. Their results showed that total IgE levels in the sera of normal Hooded Lister rats were less than 0.35 μg/ml, but these levels increased to 250–500 μg/ml at 12 days after infection. Recently, we studied the relationship between total IgE and IgE antibody against worm antigen, following the infection of Sprague-Dawley rats with *N. brasiliensis* larvae (58). The results showed that total IgE level began to increase about 10 days after the infection and reached a maximum on the fourteenth day. On the other hand, IgE antibody against worm antigen became detectable at 3 to 4 weeks after the infection, when total IgE level had already begun to decline (Fig. 2). It is apparent that the kinetics

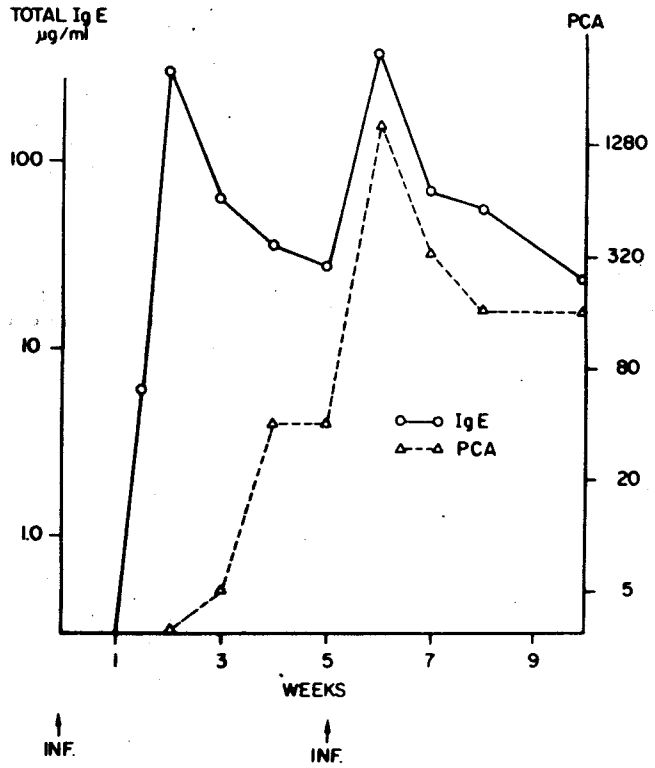

FIG. 2. Total IgE (○) and IgE antibody in the serum of a rat infected with *Nippostrongylus brasiliensis*. The IgE antibody titer (△) was determined by PCA reactions using an extract of worm as antigen. [From Ishizaka *et al.* (56).]

of the IgE antibody formation did not parallel the total IgE synthesis. Lack of correlation between total IgE and IgE antibody was confirmed in Hooded Lister rats (57).

Another interesting finding in parasite infection is that infection of rats with *N. brasiliensis* or *Fasciola hepatica* causes nonspecific potentiation of unrelated IgE antibody responses to antigens such as OA and KLH (12, 59, 126, 127). Orr and Blair (126) first described this phenomenon following the infection of OA-primed animals with *N. brasiliensis*. Bloch *et al.* (12) found that augmentation of the antibody response after parasite infection was directed only to IgE antibodies: Neither the IgG1 nor IgG2 antibody response was altered following the infection. There are some requirements for obtaining the potentiation.

First of all, rats have to be primed in such a way as to produce IgE antibody prior to the infection. Second, there should be an appropriate interval between the priming immunization with antigen and infection. In Sprague-Dawley rats, which were employed by Orr and Blair (127), an interval of 1 week to 10 days was optimal for the potentiation. Neither the infection prior to the immunization nor late infection after the primary IgE antibody response gave potentiation. This interval, however, did not appear to be critical when Hooded Lister rats were used in the experiments. Jarrett and Bazin (58) immunized these rats with OA together with pertussis vaccine and infected them 20 days after the priming for successful potentiation. The difference among the strains may be related to the fact that the primary IgE antibody response to OA in Hooded Lister rats was more persistent than that observed in the other strains. It is also known that the Hooded Lister strain show a secondary IgE antibody response to OA without adjuvant, whereas Sprague-Dawley rats fail to respond to a booster injection. In both strains, potentiation of the IgE antibody response was observed at 12 to 14 days after the infection when the total IgE increase was maximum. These results suggest that potentiation is due to nonspecific stimulus on B cells that have been programmed for IgE antibody production by previous immunization. This idea is supported by the finding of Jarrett *et al.* (59), who demonstrated that IgE antibodies against both OA and KLH were potentiated following parasitic infection if the rat had been primed with both antigens.

The potentiation of an IgE antibody response after *N. brasiliensis* infection was observed in the mouse as well (82). In this species, however, infection with parasites 5 to 14 days prior to primary immunization was most effective for potentiation, whereas the infection after the immunization was ineffective. The reasons for these differences between rats and mice are unknown at the present time.

C. Distribution of IgE-Forming Cells

Th IgE-forming cells were first detected in primate lymphoid tissues by using a fluorescent antibody technique (154). In nonatopic individuals, recurrently infected tonsils and adenoids removed by surgery possessed a large number of plasma cells that stained with anti-IgE. Some germinal centers in these tissues also stained. Bronchial and peritoneal lymph nodes contained IgE-forming plasma cells as well as germinal centers. By contrast, IgE-forming cells were scarce in spleen and subcutaneous lymph nodes.

The IgE-forming cells were detected in respiratory and gastrointestinal mucosa. In nasal mucosa, some of the plasma cells under epithelial cells stained with anti-IgE. Immunoglobulin E-forming cells were found in the bronchial mucosa especially around the mucous serous glands. In the stomach, small intestine, colon, and rectum, IgE-forming cells were observed in the lamina propria, especially around the crypts of Lieberkühn. Lymphoid cells in bone marrow, lung tissues, and peripheral blood from nonatopic individuals did not stain with anti-IgE. The distribution of plasma cells and germinal centers that stained with anti-IgE is summarized in Table I, which also shows the distribution of IgE-forming cells in monkey tissues. It would appear that the IgE-forming cells predominate in the respiratory and gastrointestinal mucosa and in the regional lymph nodes.

TABLE I
Distribution of IgE-Forming Cells in Lymphoid Tissues

Lymphoid tissues	Human[a]		Monkey[a]	
	Plasma cells	Germinal center	Plasma cells	Germinal center
Tonsil	+ ~ +++	+ ~ ++	+	++
Adenoid	+ ~ +++	+ ~ ++		
Bronchial and peritoneal	++	(+)	++	(+)
Subcutaneous lymph node	± ~ +	−	±	−
Spleen	± ~ +	−	+ ~ ++	±
Respiratory mucosa	+	−	+	−
Gastrointestinal mucosa	+ ~ ++	−	+ ~ ++	(+)[b]
Lung	−	−	−	−
Blood	−	−	nd	nd
Bone marrow	−	−	nd	nd

[a] Parentheses indicate negative in some cases; nd, not determined.
[b] Plus in Peyer's patches.